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Vol. XXXVI June, 1947 No

JUL 11 1947

SCIENTIFIC EDITION

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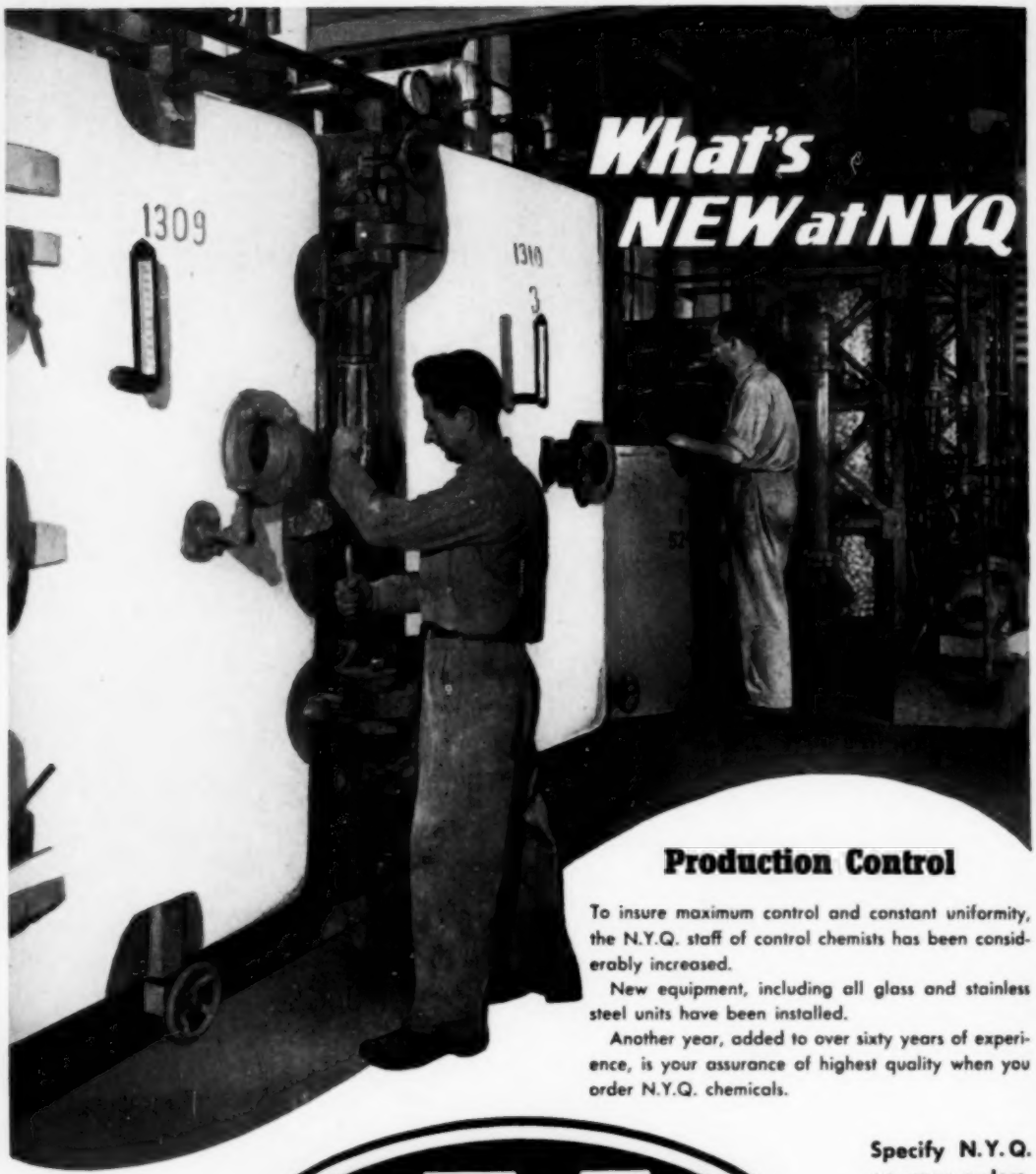

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVI — *JUNE, 1947* — NUMBER 6 — CONSECUTIVE NO. 12

Desoxyephedrine—A Review of the Literature*

By THOMAS J. HALEY

SINCE 1943 when Ivy and Goetzl (1) published their review on desoxyephedrine much additional work on its chemistry, pharmacology, and therapeutic use has been done. Several reviews have appeared in the foreign literature (2–4).

Many names have been used to designate the two isomers and the racemic mixture of the drug. Chemically it has been called β-phenylisopropylmethylamine, N-methyl phenylisopropylamine, and 1-phenyl-2-methylamino propane. "Pervitin," "D-O-E," "Desoxyn," and "Methedrine" have been used to designate the *d*-isomer, "Methyl Isonym" the *l*-isomer, and "Oxydrine" and "Desamine" the racemic mixture. The names Methyl Benzedrine, Methyl Amphetamine, and N-Methyl Amphetamine have been used for the three forms of the drug in general.

This review will attempt to present a complete report on the chemistry, pharmacology, and clinical usage of the three forms of desoxyephedrine.

* Received June 28, 1946, from the Research Division, E. S. Miller Laboratories, Inc., Los Angeles 14, Calif.

CHEMISTRY

Synthesis.—Ogata (5) first synthesized desoxyephedrine by the reduction of the condensation product of benzylmethylketone and methyl amine. Separation of the isomers from the racemic mixture obtained showed that the *d*-isomer was identical with the compound obtained by the reduction of *l*-ephedrine. Several characteristic derivatives were described (Table I).

Emde (6) produced *l*-desoxyephedrine by the catalytic reduction of *d*-pseudoephedrine.

Recently Erlenmeyer and Simon (7) produced the *dl*-compound by the reduction of 4-methyl-5-phenylthiazole with sodium in alcohol. The resulting solution was made alkaline with sodium carbonate and extracted with ether to yield *dl*-desoxyephedrine base.

Rosenmund, Krag, and Marcus (8) used another method of synthesis, reducing ephedrine in the presence of $HClO_4$ with a Pd-$BaSO_4$ catalyst. *l*-Ephedrine reacted within forty minutes to form *d*-desoxyephedrine and *dl*-ephedrine within ten minutes to form *dl*-desoxyephedrine. Many examples of the reaction involved were reported and the yields were from 60% to 90% of theoretical.

Novelli (9) employed an aromatic ketone and an aliphatic amine to obtain yields of from 50% to 70% of the racemic compound as the hydrochloride. Purification was accomplished by precipitation of the

racemate from an absolute alcohol solution with anhydrous ether.

Graham, Cartland, and Woodruff (10) reported a method applicable to the preparation of both the normal and isopropyl amines of the ephedrine series. This procedure called for Hofmann degradation of the appropriate acid amide followed by methylation to obtain *dl*-desoxyephedrine.

Identification.—Recently Hamilton, *et al.* (11) synthesized two new characteristic derivatives of *dl*-desoxyephedrine. These derivatives were obtained as a result of onium salt formation with both sulfathiazole and sulfadiazine (Table I). The *d*-isomer has been identified by converting it into a picrolonate (12).

EXPERIMENTAL PHARMACOLOGY

Blood Pressure.—Hauschild (15) reported that 10 micrograms of *d*-desoxyephedrine intravenously in cats and dogs caused a blood pressure rise almost as great as that produced by ephedrine. Doses of 50–100 micrograms caused a prolonged rise. In decapitated or vagotomized animals the effect was less. No reversal of the blood pressure effect was seen after the administration of ergotamine tartrate. Further, oral administration (stomach or duodenum) of the drug caused a prolonged rise. Tachyphylaxis was observed upon continued administration.

Seifert (16) observed an increased blood pressure and tachyphylaxis in cats after doses of 0.05 mg./Kg.

TABLE I.—PHYSICAL CONSTANTS OF DESOXYEPHEDRINE

Form	Derivative	M. P., °	B. P., 84° 13 mm.	Specific Rotation	Partition Coefficient Xylene/Water	Reference
	Base					7
	Hydrochloride	133–135	..	..	38	8
	Hydrochloride	132–133	..	..	..	14
	Hydrochloride	134	..	..	..	9
Racemic	Platinic Chloride	198–199	..	..	..	7
	Thiourea	134	..	..	..	7
	Sulfathiazole	118–120	..	..	..	11
	Sulfadiazine	187–189	..	..	..	11
Levo	Tartrate	159–160	..	−14	..	14
	Hydrochloride	170–171	..	..	..	5
	Hydrochloride	171–173	..	+14	..	14
	Hydrochloride	171–173	..	..	53	8
	Bitartrate	118–119	..	..	..	5
Dextro	Picrate	144–145	..	..	..	5
	Mercuric Chloride	152–153	..	..	..	5
	Gold Chloride	127	..	..	..	5
	Platinic Chloride	214–215	..	..	..	5
	Picrolonate	183	..	..	..	12

Analysis.—Because desoxyephedrine contains nitrogen in its molecule, the drug may be analyzed by the Kjeldahl or the Dumas methods. Both the acid salts and the free base may be determined by titration.

Dultz (12) used this latter method for the determination of the amount of the *d*-isomer contained in cadaveric material.

Richter (13) has employed a nonspecific picric acid reagent for the determination of the rate of urinary excretion of the *l*-isomer. This is a colorimetric method of great accuracy.

Novelli and Tainter (14) used the standard chloride procedure for the determination of the purity of both the *d*- and *dl*-forms of the drug.

All of the above procedures give accurate results when the drug is not combined with other materials which react under the same conditions. However, they will not determine which form of the drug has been used. There is a great need for a rapid, accurate, and specific analytical procedure for the determination of desoxyephedrine.

intravenously. Mahorny and Orzechowski (17) found that *l*-ephedrine, *d*-desoxyephedrine, and amphetamine sensitized the decapitated cat against epinephrine. Tachyphylaxis was always seen.

Raymond-Hamet (18) called this tachyphylaxis seen with *d*-desoxyephedrine a rapid immunization of the animal to the drug, thus differentiating it from a toxic side reaction.

Further investigation by Hauschild (19) showed that the blood pressure effect of all forms of desoxyephedrine was equal when equal doses (0.02 mg./Kg. intravenously) were given to cats. However, Swanson, *et al.* (20) showed that in the decerebrated and pithed dog the *l*-isomer was stronger than the *d*-isomer with the *dl*-form occupying an intermediate position in regard to pressor activity.

Graham, *et al.* (10), using the United States Pharmacopœia procedure, evaluated the pressor activity of *dl*-desoxyephedrine at 1/275 and amphetamine at 1/250 as compared to epinephrine at 1.

Riechert and Schmieder (21) compared the effect of several sympathomimetic amines on the blood

TABLE II.—EFFECTS OF INTRAVENOUSLY ADMINISTERED SYMPATHOMIMETIC AMINES ON BLOOD PRESSURE AND SPLEEN CONTRACTION IN CATS

		Normal Cat				Decapitated Cat			
		Blood Pressure		Spleen		Blood Pressure		Spleen	
Drug	Conc.	Rise, Mm.	Duration, Min.	Contraction, Mm.	Duration, Min.	Rise, Mm.	Duration, Min.	Contraction, Mm.	Duration, Min.
Epinephrine	1:160	20–70	5	16–35	8–20	..	..	10–28	10–20
Ephedrine	1:40	70	60	12	70	..	..	...	...
Amphetamine	1:40	60	30	10	30	40	20	8	20
d-Desoxyephedrine	1:40	80	30	15	60	40	70	5	70

pressure and spleen contraction in normal and decapitated cats. Their results are given in Table II.

Domenjoz and Fleisch (22) had previously shown that *d*-desoxyephedrine caused a blood pressure rise simultaneously with spleen contraction. The intravenous threshold dose was the same as for *l*-ephedrine. Further work confirmed these findings (23).

Gaede (24) applied 2.5 mg. of epinephrine to the nasal membrane of dogs, cats, and rabbits and obtained a blood pressure increase of 30 mm. with a duration of six to eight minutes. Under the same conditions 5 mg. of *d*-desoxyephedrine caused a greater blood pressure rise with a duration of one hour.

Vasoconstriction.—Domenjoz and Fleisch (25) compared the effect of amphetamine, *d*- and *l*-ephedrine, and *d*- and *dl*-desoxyephedrine upon the venous blood flow in cats under Numal or urethane anesthesia. They found that the vasoconstrictor effect was greatest with amphetamine and that *d*- and *dl*-desoxyephedrine were almost equal, while both ephedrines were much weaker.

Konzett (26) perfused the isolated hind limbs of dogs with arterialized blood and found that *d*-ephedrine, *l*-pseudoephedrine, *l*-desoxyephedrine, and *l*-Veritol quickly produced tachyphylaxis and then a marked decrease in blood pressure.

Derouaux (27) showed that *d*-desoxyephedrine exerts its hypertensive action due to peripheral vasoconstriction. Doses of 0.1 mg. intravenously markedly reduced bleeding time in rabbits.

Pulse and Cardiac Action.—Krause (28) found that the therapeutic dose of *d*-desoxyephedrine in dogs was 0.1–0.3 mg./Kg. subcutaneously. An increase in the pulse wave was seen with dose, while larger doses produced a vagus pulse. Subcutaneous administration of 150 mg. to horses and cattle caused an improved fullness of the pulse, an increase in heart tone, and a cardiac apex-bear.

Respiration.—Novelli and Tainter (14) reported that 2 mg. of *d*-desoxyephedrine intraperitoneally administered to rats caused hyperventilation with an increase in the rate and depth of respiration. This effect persisted at a slightly decreased value for several hours.

Blume and Zollner (29) confirmed these observations, showing that doses of 0.05–0.1 mg./Kg. of *d*-desoxyephedrine had no effect while doses of 0.5–3 mg./Kg. subcutaneously increased the rate and to a lesser extent the volume of each inhalation. In guinea pigs subcutaneous doses of 0.05–10 mg./Kg. caused a decrease in lung volume and an increase in rate while doses of 20 mg./Kg. increased both the rate and volume of each inhalation.

The increased volume seen may have been due to the bronchiodilator effect of desoxyephedrine. Graham, *et al.* (10) showed that *dl*-desoxyephedrine increased the rate of bubble formation in the perfused rabbit lung preparation. Amphetamine under the same conditions decreased bubble formation thus showing a constrictor action.

Riechert and Schmieder (21) found that in rats epinephrine and Sympatol after cocaine or in lethal doses caused lung edema. *d*-Desoxyephedrine, amphetamine, ephedrine, and Veritol under the same conditions did not have this effect.

Genitourinary Effects.—Santi (30) reported that *d*-desoxyephedrine was more active than amphetamine in increasing the tone of the isolated rabbit uterus. The action was thought to be directly upon the muscle. Similar results were reported by Mahorny and Orzechowski (17).

Neuweiler (31) found that although both *d*-desoxyephedrine and ephedrine relaxed the guinea-pig uterus, epinephrine was ten times more potent that either of them. He also found that after daily injections of 0.6–0.8 mg./100 Gm. of *d*-desoxyephedrine in the rat, the rate of estrus cycles was decreased or complete anestrus resulted. However, corpora lutea persisted.

Springorum (32) found that *d*-desoxyephedrine increased renal blood flow thereby increasing the secretion of urine.

Intestinal Effects.—Hauschild (15) reported that *d*-desoxyephedrine counteracted pilocarpine or arecoline spasms of the isolated rabbit intestine at a concentration of 1:100,000.

Reiser (33) found that *d*-desoxyephedrine at a concentration of 1:700,000 caused peristaltic standstill of the rabbit small intestine in forty seconds and the effect lasted for nine minutes. At a concentration of 1:140,000 the action began in twenty seconds but had a shorter duration. However, the effect repeated itself without the addition of more drug. Amphetamine under the same conditions at a concentration of 1:700,000 showed the same effect in one hundred thirty seconds with a duration of eleven minutes.

Hauschild (19) later reported that, with papaverine rated at 1 in overcoming spastic contraction of the isolated guinea-pig intestine caused by Doryl or barium chloride, *d*-desoxyephedrine and amphetamine were both rated at 0.2.

Neuweiler (31) found that 0.75 mg. of the same isomer inhibited the isolated rabbit intestine, while 1.5–3 mg. stimulated it.

Niessing and Orzechowski (34) studied the effect of *d*-desoxyephedrine, Veritol, epinephrine, ephedrine, Sympatol, and amphetamine on the cell structure of the guinea-pig omentum. They showed that epinephrine, *d*-desoxyephedrine, ephedrine, amphetamine, Sympatol, and Veritol in that order caused contraction of the epithelial cells of the tissue.

Body Temperature.—Kiessig (35) administered subcutaneous doses of 0.03 mg./Kg. of *d*-desoxyephedrine and 0.05 mg./Kg. of amphetamine to mice and obtained equal rises in temperature. Also 2.5 mg./Kg. intravenously of *d*-desoxyephedrine gave a greater temperature rise in dogs than 5.0 mg./Kg. of amphetamine. Further, toxic doses of *d*-desoxyephedrine (2.5–5 mg./Kg.) in dogs raised their temperature from 39° to 44°.

Hauschild (15) previously had reported this hyperthemia after lethal doses of *d*-desoxyephedrine in rats.

This temperature-elevating effect appears to be a definite property of this type of amine. Warren and Werner (36) observed a definite hyperthermia after the subcutaneous administration of *l*-ephedrine and *dl*-1-methylamino-2-phenylpropane.

Analgesia and Anesthesia.—Kiessig and Orzechowski (37) electrically stimulated the tooth pulp of dogs after the intravenous administration of amphetamine and *d*-desoxyephedrine. They found that the latter had a greater effect in decreasing pain sensitivity. Further, they checked this action with the mouse tail technique and confirmed their results.

In a further extension of this work Orzechowski (38) showed that 3% ephedrine, 3% Veritol, and 2% amphetamine had a more rapid and more prolonged local anesthetic action than 2% *d*-desoxyephedrine when applied to the frog sciatic nerve preparation. This procaine-like action could be abolished by washing with Ringer's solution.

Kiessig (39) obtained similar results upon the rabbit's cornea. He reported that *l*-ephedrine, Sympatol, Veritol and amphetamine all in 1% solution were more effective than 1.5% *d*-desoxyephedrine. Increased concentrations of all but ephedrine caused irritation.

Metabolism.—Thaddea, Friedrichs, and Kohns (40) analyzed the muscle and liver of dogs, rats, and rabbits two and one-half hours after the subcutaneous administration of 0.5 mg./Kg. of *d*-desoxyephedrine. They found that there was no effect on either muscle or liver glycogen. Further, the drug had no effect on blood sugar or blood lactic acid in the normal rat and rabbit, or on the blood sugar of the depancreatectomized dog. Also neither glucosuria nor creatinuria were seen.

Elimination.—The elimination of desoxyephedrine after administration to animals has not been determined. However, the drug should be eliminated in a manner similar to that reported for amphetamine, its primary analogue.

Beyer (41) showed, with five dogs, that amphetamine excretion was decreased about 35% after the daily administration of 200–400 mg. of ascorbic acid. Previous studies by Beyer and Skinner (42) had shown that carbon-tetrachloride-poisoned dogs excreted amphetamine quantitatively within twenty-four hours. Beyer and Lee (43) in an extension of this work showed that hydrazine produced the same quantitative excretion of amphetamine.

Enzyme Systems.—Many papers have reported the action of various enzyme systems on sympathomimetic amines. Beyer (44) has shown that molecular configuration determines the mode of inactivation by amine oxidase and phenol oxidase. Amine oxidase in the presence of oxygen can oxidize phenylpropylamines having the amino group on the terminal carbon atom of the side chain. Phenol oxidase requires the presence of one or two hydroxyl groups on the benzene ring in order to oxidize this type of amine.

Blaschko, Richter, and Schlossmann (45) found that phenylisopropylamine and its derivatives were not oxidized by amine oxidase. Later Blaschko (46) showed that these compounds were amine oxidase inhibitors. Thus amine oxidase would not oxidize desoxyephedrine.

Steensholt (47) reported that suprarenal medulla tissue, which contains monoamine oxidase, showed a marked increase in oxygen uptake in the presence of both ephedrine and amphetamine. In this report no mention was made of the ascorbic acid content of the tissue used even with the reports of the large amounts of that vitamin in the suprarenal gland.

The presence of ascorbic acid might explain the oxygen uptake because Beyer (41) has shown that ascorbic acid can deaminate amphetamine both *in vitro* and *in vivo*. Further, he has shown that the ascorbic-dehydroascorbic acid system can deaminate amphetamine as well as other sympathomimetic amines (48).

Similar enzyme studies have shown that amphetamine caused an increase in the co-carboxylase synthesis by pigeon-liver slices (49). Also succinic oxidase of rat muscle and liver was stimulated even after inhibition by α-tocopherol. The oxygen uptake increased and the increased metabolic activity could be utilized for co-carboxylase synthesis from thiamine and pyrophosphate (50).

Analeptic Action.—Hess (51) observed that intraperitoneal doses of 25, 50, 75, and 100 micrograms/100 Gm. of *d*-desoxyephedrine had an awakening action in alcohol intoxicated rats. The larger doses had a greater effect. However, in chloralose narcosis in rats the same drug in doses of 10, 75, and 100 micrograms/100 Gm. intraperitoneally prolonged the narcosis.

Krause (28) reported that in morphine or mor-

phine-pernocton narcosis in animals *d*-desoxyephedrine had a definite arousing action.

Koshtoyants and Mitropolitanskaya (52) found that 5–10 mg. of *d*-desoxyephedrine given to hamsters and hedgehogs one and one-half months before the end of hibernation caused a wakeful state combined with an unusual motor activity for a period of two to four hours. Afterwards the animals promptly fell asleep.

Hauschild (53) observed that *d*-desoxyephedrine in doses of 0.1–0.2 mg. in 20–25 Gm. mice counteracted the hypnotic effect of 2 mg. of ethylallylbarbituric acid. Jacobsen, *et al.* (54) rated *d*-desoxyephedrine above amphetamine in analeptic activity using the same test.

Trevan (55) found that both metrazol and picrotoxin were better analeptics than desoxyephedrine, because the sympathomimetic and other side effects of the latter reduced the margin of safety.

CLINICAL USAGE

General Considerations.—Eichholtz (60) has tabulated and compared the effects of *d*-desoxyephedrine with those seen with opium, cannabis, alcohol, caffeine, nicotine, and a series of sympathomimetic amines. The intensity of action was shown to depend upon the configuration of the drug.

Schulz and Deckner (61) have shown that *d*-desoxyephedrine has a direct effect upon circulation, blood pressure, pulse frequency, and depth of respiration, but no direct effect on metabolism. It increased the functional activity of the cerebral cortex and brain stem, elicited autonomic activity in the ganglia, and changed the chronaxie values. Further, a central action in addition to a spasmolytic action was assumed for the reduction in the gastric peristalsis seen.

This isomer has been shown to produce the most rapid onset of effect, act for the longest period of time, and stimulate the greatest degree of motor activity of all the cephalotropic amines. Insomnia and central irritation were often seen but euphora was often absent. Davidoff (62) compiled these results from research on sixteen human subjects, who received oral doses of 10 mg. of either *d*- or *dl*-amphetamine or *d*-desoxyephedrine.

TABLE III.—LD_{50} DOSE OF DESOXYEPHEDRINE IN ANIMALS

Animal	Isomer	Route	Dose, Mg./Kg.	Reference
Rat	Dextro	Intraperitoneal	15–25, av. 17	Hauschild (15)
Rat	Dextro	Oral	37	Hauschild (15)
Rat, large	Racemic	Intravenous	20	Graham, *et al.* (10)
Rat, large	Racemic	Intraperitoneal	54	Haley and Flesher (56)
Mouse	Dextro	Intraperitoneal	22	Richards (59)
Mouse	Dextro	Subcutaneous	9–20, av. 10	Richards (59)
Mouse	Dextro	Subcutaneous	15–150	Kiessig (35)
Mouse	Dextro	Oral	20–90	Richards (59)
Mouse	Racemic	Intraperitoneal	61	Haley and Flesher (56)
Mouse	Racemic	Oral	143	Haley and Flesher (56)
Rabbit	Dextro	Intravenous	15–25	Kiessig (35)
Rabbit	Dextro	Intravenous	15–30	Richards (59)
Rabbit	Dextro	Subcutaneous	7.5	Richards (59)
Dog	Dextro	Subcutaneous	Above 20	Krause (28)
Frog	Dextro	Lymphsac	1000	Wulff (58)

Central Motor Stimulation.—Hauschild (15) found that doses of 0.03 mg./100 Gm. in mice caused an increased activity as recorded in the jiggle cage. Novelli and Tainter (14) using the same method found that rats were stimulated as follows: *d*-desoxyephedrine 0.0625–16 mg., duration seven and one-half hours; *dl*-desoxyephedrine 0.125–8 mg., duration six and one-half hours; and *l*-desoxyephedrine 1–128 mg., duration twenty-one hours. When compared with *d*-amphetamine, *d*-desoxyephedrine had a lower threshold dose and almost the same duration of action.

Toxicity.—In rats and mice the symptoms of toxicity seen were piloerection, unruliness, excitation, nasal hemorrhages, convulsions, and death by respiratory paralysis (15, 18, 33, 56). In dogs symptoms of toxicity were circus movements of the forelegs and intensified respiration (27). Heubner and Stuhlmann (57) stated that on the basis of their rat toxicity work, *d*-desoxyephedrine was slightly more toxic than amphetamine. Wulff (58) found that toxic doses of *d*-desoxyephedrine in frogs caused paralysis by a direct action on the spinal cord. All the LD_{50} doses of the three forms of desoxyephedrine in various animals are given in Table III.

Vascular and Respiratory Effects.—The effects of desoxyephedrine on the vascular and respiratory systems of humans follow the same pattern as that seen in animals. One-half hour after intramuscular injection of 15 mg. of *d*-desoxyephedrine into healthy humans, Berseus (63) observed that the heart minute volume was increased to 39% over normal. After one to one and one-half hours the increase was only 20%. In five of the seven subjects tested neither the ventilation nor the respiratory quotient was increased. However, oxygen consumption was increased.

Matthes (64) found that intravenous injection of 9 mg. of *d*-desoxyephedrine caused a prolonged peripheral vasoconstriction and a rise in systolic blood pressure which returned to normal after one hour. Doses of 3 and 1 mg., respectively, had the

same effects but the latter dose had no central nervous system effects. In all the cases studied the respiration showed little or no change. Changes in the pulse rate and the production of tachycardia were seen after the injections. Intramuscular injection of 15 mg. of the drug gave the same results after a four to five minute delay and oral administration of 15 mg. was similar but the action was delayed for twenty minutes. The investigator concluded that subcutaneous or oral administration was as effective as intravenous injection. Table IV gives the results of this work.

TABLE IV.—EFFECTS OF SYMPATHOMIMETIC AMINES IN HUMAN SUBJECTS[a]

Mg. Dose and Drug	Blood Pressure Rise, Mm.	Respiration	Ear Volume	Finger Volume	Ear Oxygen Saturation	Finger Oxygen Saturation	Ear Temperature	Finger Temperature
9 D.	120–165 tachycar.	No great change	Dec.	Inc., then dec.	Dec.	Inc., then dec.	Dec.	Dec.
3 D.	140–165 tachycar.	No great change	Dec.	Dec.	Dec.	Dec.	Dec.	Dec.
8 V.	140–165 max. 3 min. tachycar.	Inc. 1 min. after injection	Inc., normal, then dec.	Dec.	Dec.	Dec.	Dec.	Dec.
20 Sup.	130–155 max. 3 min. tachycar.	Hyperventilation, then irregular	Inc., normal, then inc.	Dec.	Dec.	Dec.	Dec.	Dec.
30 Eph.	140–180, 160 in 5 min. tachycar.	No great change	Dec.	Inc., then dec.	Dec.	Inc., then dec.	Dec.	Dec.
20 A	140–180 tachycar.	No great change	Dec.	Inc., then dec.	Dec.	Inc., then dec.	Dec.	Dec.
30 Sym.	155–210 rapid recovery	Inc.	Dec.	Inc. then dec.	Dec.	Inc., then dec.	Dec.	Dec.
0.04 Epi.	160–210 rapid recovery	Inc.	Dec.	Inc., then dec.	Dec.	Inc., then dec.	Inc., normal in 2 min.	Dec., then normal

[a] D. = desoxyephedrine, V. = Veritol, Sup. = Suprifen, Eph. = ephedrine, A. = amphetamine, Sym. = Sympatol, and Epi. = epinephrine, tachycar. = tachycardia, inc. = increased, dec. = decreased.
Doses of epinephrine (0.003, 0.01, and 0.05 mg.) were similar to that of 0.04 mg.

Kohler (65) states that, in normal humans, 5 mg. of *d*-desoxyephedrine acts like epinephrine and is synergistic with it in its blood pressure effects. Both drugs cause hyperglycemia and are antagonistic to insulin. However, in general blood sugar is not modified to any great degree by *d*-desoxyephedrine.

Franke (66) confirmed these effects in 30 young and old men. He found that 6 mg. of the drug orally caused a systolic blood pressure rise of 10–20 mm. over a period of two to three hours. Doses of 15 mg. had a similar effect. In both instances the pulse rate and blood sugar values were unchanged.

Golla, Blackburn, and Graham (67) could find no significant difference in the effect of *d*-desoxyephedrine and amphetamine on blood pressure and pulse rate.

Elimination.—In a study of the elimination of amines orally administered to humans, Richter (13) found that although the kidney was able to concentrate considerable amounts of the drugs (amphetamine 10–13 micrograms per cc. and *l*-desoxyephedrine 5 micrograms per cc.) the excretion rate was slow (Table V). He concluded that *l*-desoxyephedrine was quantitatively excreted.

However, Beyer (68) did not confirm these findings in regard to amphetamine. Using oral doses of 10, 20, and 30 mg. in fifteen human subjects, he found that 22.1–66.3% of the drug was excreted in the urine within forty-eight hours.

Further investigation into the mode and rate of excretion of both of these drugs should clarify the situation.

Analeptic Activity.—The analeptic effect of *d*-desoxyephedrine in antagonizing ethylallylbarbituric acid in humans is similar to that in experimental animals (19). However, Jacobsen, *et al.* (54) rated amphetamine higher than *d*-desoxyephedrine in this activity in humans. Warstadt (69) found that oral administration of *d*-desoxyephedrine was nearly as effective as by the subcutaneous route. Jacobsen (70) attributed this analeptic action to irritation of the central nervous system.

Neumann (71) reported that central stimulation was the principal action of *d*-desoxyephedrine but side effects were seen after large doses. These ef-

TABLE V.—PER CENT RECOVERY OF AMINES FROM THE URINE IN HOURS

Drug	Dose as the Base, Mg.	12 Hours, %	24 Hours, %	48 Hours, %
Ephedrine	26	77	97	97
	52	59	92	99
	104	59	77	82
Amphetamine	14.7	..	40	70
l-Desoxyephedrine	8	..	36	56

fects were constipation, increased sweat secretion, palpitation of the heart, and insomnia.

Pullen (72) used this action to combat alcoholic intoxication. Oral doses of 6–9 mg. of *d*-desoxyephedrine were used. Increased arterial pressure was seen with the smaller dose. Later he reported that 9–15 mg. intramuscularly had a prolonged analeptic action (73).

Siegmund (74) used *d*-desoxyephedrine to counteract alcoholic intoxication. Oral doses of 6–12 mg. of the drug were more effective as premedication but if taken during the intoxication were still effective.

Postma (75) used this analeptic action therapeutically to combat barbital poisoning. Doses of 81 and 90 mg. of *d*-desoxyephedrine were given by stomach tube for two days. Jecel (76) also reported that 15–25 mg. of this isomer counteracted barbiturate narcosis.

Work Capacity and Antifatigue Effects.—Jacobsen (77) could detect no improvement in the intelligence scores of experimental subjects after premedication with *d*-desoxyephedrine.

Simonson and Enzer (78) reported that 5–7.5 mg. of *d*-desoxyephedrine orally was less effective than 10–15 mg. of amphetamine when tested by the fusion of frequency flicker and the motor-frequency tests. However, the former did increase the endurance of the motor centers.

Pellmont (79), using a large number of physical and mental tests, found that 6 mg. of *d*-desoxyephedrine orally relieved both the mental and physical fatigue of ten subjects but had a greater effect in the latter.

A report of twelve young men on a short winter course of training showed that *d*-desoxyephedrine had a stimulatory effect upon blood pressure, pulse rate, and recovery time. Oral pre- and post-medication were used by De Puoz (80) but he did not recommend the use of the drug for this purpose.

Lehmann, Straut, and Szakáll (81) administered 5–15 mg. of *d*-desoxyephedrine orally to three young men, twenty-one to twenty-six years of age, and found that they could work to the limit of maximum efficiency but after exhaustion their efficiency was lowered considerably. The conditions of this experiment were varied considerably and controls were maintained by having the subjects go through the same tests without medication. No metabolism changes were noted after medication either during work or rest.

Heyrodt and Weissenstein (82) found that the intramuscular injection of 15 mg. of the same drug twenty minutes before testing, increased the running time of the experimental subjects.

To test the antifatigue effects of *d*-desoxyephedrine, Alwall (83) gave 700 soldiers, twenty to twenty-two years of age, the drug on the last morning of a three-night exertion. There was a reduction or elimination of the subjective fatigued appearance and an improvement in the mental attitude. Further work with 1400 soldiers showed that both amphetamine and *d*-desoxyephedrine improved their ability to march and do mathematical calculations (84).

In a similar type of experiment Foltz, Ivy, and Barborka (85) found that *d*-desoxyephedrine acted as a mental stimulant in fatigued trained subjects. However, the effects were more pronounced if the drug was given to the nonfatigued subject than if given after exhaustion.

Psychic and Psychomotor Effects.—Flugel (86) stated that *d*-desoxyephedrine was distinctly beneficial in depressed psychopathic states.

Graf (87), using tests of psychic and psychomotor functions, showed that this isomer, in oral doses of 9–15 mg., eliminated the feeling of fatigue and caused a loss of inhibitions. Further, although it lessened the ability to concentrate, increased activity and efficiency occurred because of the relief of nervous tension. Schulz and Deckner (61) confirmed these results, stating that the psychic effect resulted in an increase in the capacity for concentration. This resulted in an increased rate of work without faulty performances. However, Lemmel and Hartwig (88) found that 3–6 mg. of the drug orally improved psychomotor activity but 9 mg. was likely to decrease concentration and induce errors.

Warstadt (69) found *d*-desoxyephedrine effective in the treatment of apathetic psychopaths and in the milder types of depression. Catatonic stupor was regularly benefited. Favorable effects were seen in the treatment of encephalitic parkinsonism but in arteriosclerotic parkinsonism and severe depressions the drug exerted a very unfavorable action.

Eaton (89) found that, in narcolepsy, treatment with *d*-desoxyephedrine was preferred by the patients.

Heat Tolerance.—Heat tolerance was improved by *d*-desoxyephedrine but the action was due to a psychic change so that the drug was not a true prophylactic (90).

Local Effects on the Respiratory Tract.—Local application of a mixture of sulfathiazole with 0.125% of *dl*-desoxyephedrine to the nasal mucosa showed a definite prolonged vasoconstriction without other toxic side effects. Ciliary motility was favorably influenced and there was no histological evidence of injury to the mucous membrane (91). Dautrebande *et al.* (92) observed an increased lung volume after inhalation of desoxyephedrine aerosols.

Effects of Blood Pressure during Anesthesia.—Dodd and Prescott (93) investigated the effect of *d*-desoxyephedrine on the hypotension seen during anesthesia and reported that the drug had a favorable effect. In an extension of these observations, they found that doses of 15–30 mg. intramuscularly or 10–20 mg. intravenously plus a depot dose of 10–20 mg. intramuscularly restored blood pressure to normal levels in two to eighteen minutes. The pressor effect was maintained for several hours after the operation. From a clinical standpoint they considered *d*-desoxyephedrine superior to epinephrine, ephedrine, and Veritol in maintaining blood pressure (94).

Prescott (95) continued these clinical studies using groups of 20 patients each for the comparison of pressor response to *d*-desoxyephedrine, Paredrine, Neo-synephrine, and Veritol. The patient had to have a severe fall in blood pressure (to 80 mm. systolic or to 10 mm. pulse pressure) to be used in his study. On the basis of the results obtained, he concluded that *d*-desoxyephedrine was superior to the three other drugs studied.

Anderson (96) investigated the effect of *d*-desoxyephedrine, ephedrine, and Neo-synephrine on the maintenance of blood pressure during spinal anesthesia in 449 patients. He concluded that *d*-desoxyephedrine was the most effective pressor drug used and that its effect of elevating the mood postoperatively was a distinct advantage.

Dripps and Deming (97) compared the vascular effects of *d*-desoxyephedrine, ephedrine, Paredrine, and Pitressin-ephedrine in 2500 cases of spinal anesthesia. They also found *d*-desoxyephedrine to be more effective, act more quickly, and maintain blood pressure longer than the other drugs studied.

Hudon and Paradis (98) investigated the effect of *d*-desoxyephedrine in 39 cases of operative shock and found that the drug's peripheral action aided the capillaries in regaining their tone, thus restoring the volume of circulating blood. They employed the drug in Pentothal, ether, cyclopropane, nitrous oxide, Pentothal-curare, and chloroform anesthesias without any toxic side effects.

Idiosyncrasy and Toxicity.—Agnoli and Galli (99) and Muntner (100) have reported two cases of sensitivity to *d*-desoxyephedrine after doses of 12 and 15 mg., respectively. Urticaria occurred in one case and may have been due to excessive dosage.

Issekutz (101) took 200 mg. *d*-desoxyephedrine with coffee and alcohol during one evening while a companion took 60 mg. of the drug. Both had the same symptoms: sleeplessness, restlessness, weakness, mydriasis, some nausea and vomiting, dyspnea, tachycardia (rate 150 per minute), and arrhythmia. However, even these doses were not fatal.

Dittmar (102) reported a case where a thirty-two year old patient had been taking 90 mg. of *d*-desoxyephedrine daily for a prolonged period. The toxic symptoms seen were acute circulatory insufficiency and numerous central nervous phenomena. It was believed that cerebral anoxia caused them.

Carratala and Calzetta (103) reported that after ingestion of 30 mg. of *l*-desoxyephedrine, a patient showed signs of poisoning in two hours. These signs were deep, irregular respiration, arrhythmic pulse, agitation, muscular twitching, and periods of unconsciousness. Delirium and hallucinations appeared later. General weakness lasted for a long time during the recovery period but the acute symptoms lasted only six days.

Tolerance and Addiction.—Greving (104) has stated that tolerance to *d*-desoxyephedrine leads to increased dosage resulting in hyperglycemia, increased metabolic rate, erythrocytosis, hypocalcemia, and cardio-vascular disturbances. Wolff (105) has also reported abuses in the use of both desoxyephedrine and amphetamine.

Hansen (106) found that addiction occurred after tolerance to *d*-desoxyephedrine had developed. This excessive use led to chronic intoxication resulting in dehydration and a loss in body weight. Abstinence symptoms were not pronounced but the risk of recidivation was great.

SUMMARY

A review of the chemistry, experimental pharmacology, and clinical usage of desoxyephedrine has been presented. Most of the work reported has been concerned with the *d*-isomer. The therapeutic usefulness of this isomer has been well established. The *l*-isomer has not been investigated sufficiently; so its exact place in therapeutics still remains to be determined. Some work has been done on the pharmacology and therapeutic use of the racemic mixture and most investigators have concluded that it is slightly less effective than the *d*-isomer. Further investigation of racemic desoxyephedrine will undoubtedly determine its therapeutic usefulness.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

d-Quercitol
Chondroitin sulfuric acid
Convallamarin
Adonidin
Quebrachitol
Indoxyl potassium sulfate
2,6-Dimethoxy-4-propylphenol
Nucleosidase
Nucleotidase
Polynucleotidase
Dipeptidase
Aminopolypeptidase
Porphyrexide
Porphyrindine
Pyrithiamin
Thiolhistidine
Ergothioneine
Kynurenic acid
Kynurenine
2-Methoxy-4-vinylphenol

Iodimetric Titrimetric Determination of Ascorbic Acid*

By JANE ADAMS, MARGARET ACKER, and H. A. FREDIANI†

The iodimetric method for ascorbic acid determination is recommended for routine testing. Excellent reproducible equivalent points, in colored as well as colorless solutions, may be obtained by the use of a vacuum tube potentiometer (titrimeter). The polarized platinum-platinum electrode system serves best in this titration. With a polarizing current of 1.5 microamperes (at 1 volt) the equivalent point is sufficiently sensitive at a meter setting of −0.25 volts to enable the use of 0.01 *N* iodine solutions.

THE EXTENSIVE current literature (2) on the determination of ascorbic acid indicates the widespread interest in methods of analysis for this vitamin. The apparent difficulties encountered in applying polarographic (3) or potentiometric (4, 8) methods to this determination have left modifications of the colorimetric method suggested by Mindlin and Butler (5) based on the 2,6 dichlorophenol indophenol procedure of Tillmans and co-workers (10) as the preferred methods (7).

It recently having become necessary in this laboratory to analyze large numbers of routine samples for added ascorbic acid content on a colored pharmaceutical product it was decided to objectively review the currently known methods before choosing one for routine work. Since the ascorbic acid to be used was to be initially assayed by the official U. S. P. (11) method utilizing a visual iodimetric titration, this method was investigated potentiometrically and found to be admirably adaptable to the problem at hand. With the high sensitivity vacuum tube potentiometers currently available, a direct titration was found feasible obviating the back-titration recommended by Stevens (9). An unquestionable one drop equivalent point with iodine solutions as dilute as 0.01 *N* indicates the method to be applicable over a wide range of ascorbic acid concentrations. The use of unstable dye solutions is rendered unnecessary and the adaptability to routine determinations enhanced.

* Received Dec. 10, 1946, from Eimer and Amend, New York City.

† Present address: Merck and Co., Rahway, N. J.

EXPERIMENTAL

Various electrode pairs were investigated to determine whether the apparent lack of sensitivity indicated by Kirk and Tressler (4) could be overcome. Figure 1 clearly indicates that for the calomel-tungsten, platinum-tungsten, and calomel-platinum electrode systems an e. m. f. change of but 0.05 to 0.15 volt could be expected at the equivalent point. Use of a platinum-platinum system wherein a polarizing current of 1.5 microamperes (at approximately 1 volt) was utilized showed a clear-out e. m. f. change of 0.68 volt indicating that the desired sensitivity could be expected under these conditions. The curves in Fig. 1 were drawn from data obtained by titrating known amounts of ascorbic acid in solution with 0.1 *N* iodine. Starch solution indicator was initially added so as to compare the visual with the potentiometric end points. It was interesting to note that with a polarizing current of 1.5 microamperes the two end points coincided exactly. A single drop of iodine caused the "magic eye" to open completely and the blue coloration to appear. This was reversible since an added drop of equivalent strength ascorbic acid solution caused the "eye" to close immediately and the blue coloration to disappear. The potentiometric sensitivity was retained when 0.01 *N* iodine was used as titrant although the visual sensitivity declined to the point where 4 drops of added reagent became essential. Initially a rather high polarizing current of 15 microamps (approx. 1 volt) was employed. Although the e. m. f. change at the equivalent point was of the same order of magnitude with the high and low currents, it was found that the potentiometric end point was more sluggish at the higher currents and did not appear until an excess of from 0.2 to 0.3 ml. of iodine (visual observation) was present. Further investigation is being carried on along these lines.

Recommended Method.—Based on the information gained from the preliminary electrode pair studies the following procedure was then used. Sufficient metaphosphoric acid (2%) was added to the sample to be titrated to reduce effects of air oxidation as recommended by Musulin and King (6) and Fujita and Iwotake (1). A 250-ml. Pyrex beaker

was used for titration vessel. Sufficient distilled water was added to have a final volume of 100–200 ml. so that the stirring electrodes remained covered with solution while rotating. The Fisher titrimeter (Fig. 2) was then calibrated so that the potentiometer dial read from 0 to −1 volt. The instrument dial was then set at a reading of 75 (−0.25 volt). At this point the "eye" remains completely closed. The standard iodine solution was then added until a single drop caused the "eye" to open permanently. Standard iodine solutions of 0.1, 0.05, or 0.01 *N* were used, depending upon the ascorbic acid content of the unknown, so that from 20 to 30 ml. of titrant were employed.

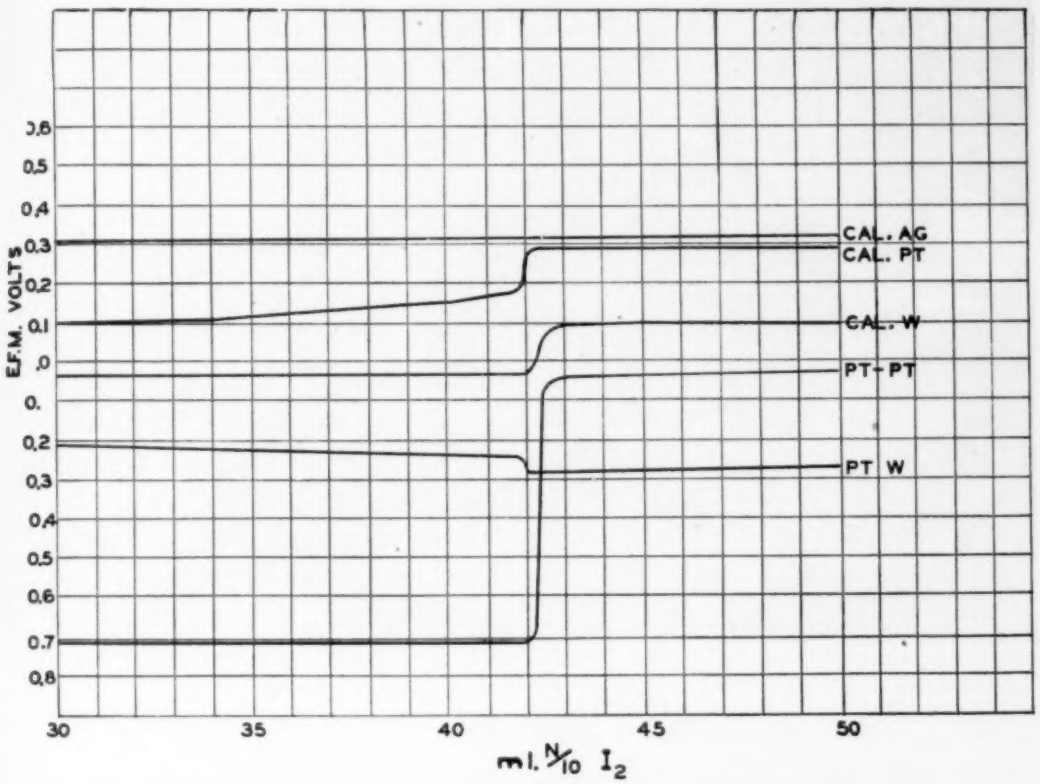

Figure 1

TABLE I.—ASCORBIC ACID DETERMINATION IN GRAVIMETRICALLY PREPARED STANDARDS

Original Sample, Gm./L.	Taken, Mg./Ml.	Found, Mg./Ml.
7.3	7.32	7.32
7.3	7.32	7.30
7.3	7.32	7.32
7.3	7.32	7.30
7.3	7.32	7.30
9.9	4.95	4.95
9.9	4.95	4.95
9.9	4.95	4.93
4.95	2.47	2.46
4.95	2.47	2.45
4.95	2.47	2.46
2.47	2.47	2.38
2.47	2.47	2.38
2.47	2.47	2.41
0.99	0.49	0.42
0.99	0.49	0.47
0.99	0.49	0.42
0.01	0.002	0.002
0.01	0.002	0.002
0.01	0.002	0.002

TABLE II.—ASCORBIC ACID CONTENT OF DARK COLORED PHARMACEUTICAL PRODUCT

Ascorbic Acid (Total)	
Added, Mg.	Found, Mg.
0.00	0.00
0.00	0.02
7.30	7.28
7.30	7.28
7.30	7.26
7.30	7.28
10.90	10.82
10.90	10.87
10.90	10.82
10.90	10.86
18.20	18.13
18.20	18.15
18.20	18.15
18.20	18.12

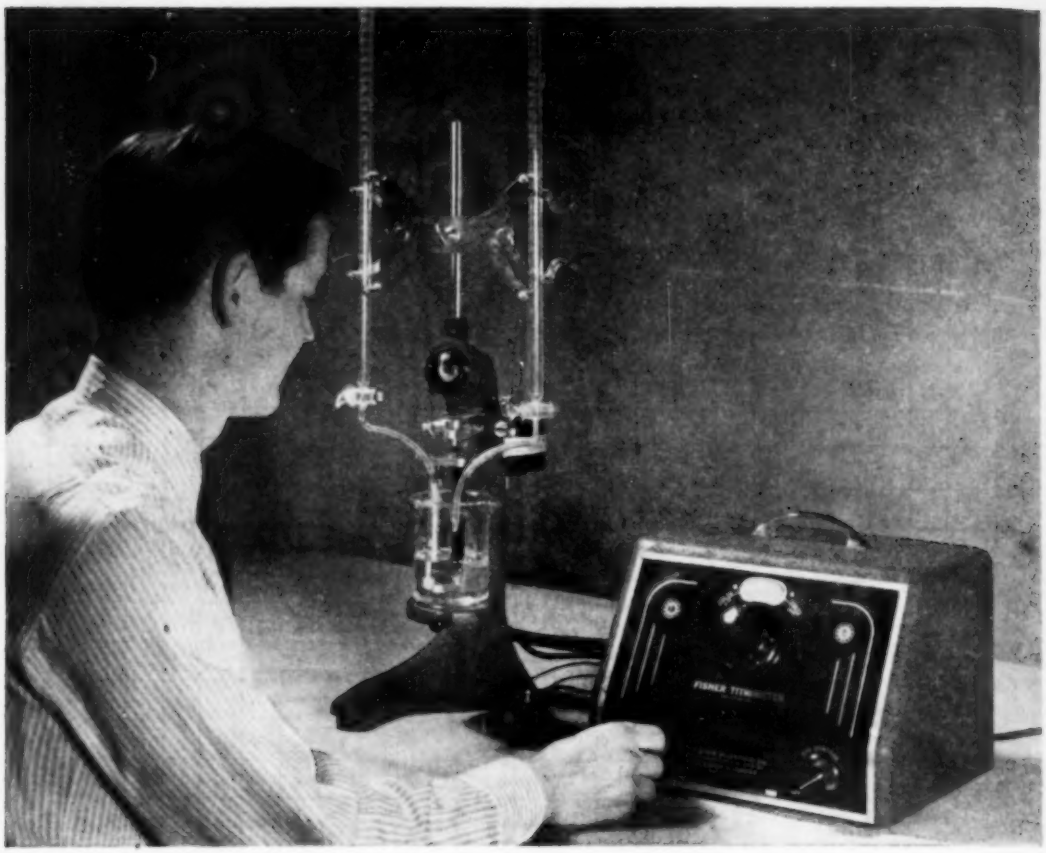

Figure 2

DISCUSSION

Table I indicates the reproducibility attainable in the potentiometric titration of buffered ascorbic acid solutions under the above conditions. The iodine solutions used were initially standardized against Bureau of Standards arsenious acid.

The commercial pharmaceutical which was to be controlled contained, in addition to the added ascorbic acid, caramel (sugar coloring), potassium iodide, and two fluid extracts. To ascertain whether any iodine oxidizable material was present in addition to the ascorbic acid, blanks were initially determined on experimental samples, from which the ascorbic acid was omitted. A zero blank was obtained.

These experimental samples were enriched by the addition of known quantities of ascorbic acid and were then analyzed. The results are summarized in Table II.

CONCLUSION

Potentiometric means for equivalent point detection permits the use of the iodimetric method for ascorbic acid determination in colored samples. The advantages of the iodine titration method, aptly discussed by Stevens (9), are retained while the double back-titration found necessary to facilitate equivalent point detection is eliminated.

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Pharmacology of Itaconic Acid and Its Sodium, Magnesium, and Calcium Salts*,†

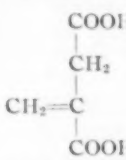
By MURRAY FINKELSTEIN and HARRY GOLD with the technical assistance of C. ADELAIDE PATERNO

Acute and chronic toxicity studies of itaconic acid (methylene succinic acid) and its sodium, magnesium, and calcium salts using cats have been made. The effects of these compounds when injected intravenously and administered orally are reported. Comparison with sodium chloride indicates that sodium itaconate has the higher molecular toxicity. This is believed, but not definitely established, to be due to the base content of the itaconate. There is indication that the itaconate ion is substantially inert.

THE METABOLISM of methyl derivatives of the lower fatty acids was recently the subject of study by Carter, Osman, Levine, and Gamm (1), and Emmrich (2). It appears that the methyl group in the β-position retards oxidation, and that unsaturated intermediary products arise during their metabolism. Emmrich obtained evidence suggesting that methylenesuccinic acid (itaconic acid) may be an intermediary product in the metabolism of the methylated 4-carbon dicarboxylic acids, and indicated that its failure to appear in the urine may result from its rapid degradation. He recovered nearly 40 per cent of a large dose of methylenesuccinic acid in the urine of a dog.

The present report deals with the pharmacology and toxicology of itaconic acid, and its sodium, magnesium, and calcium salts. The materials[1] used were anhydrous itaconic acid (99.9%), anhydrous disodium itaconate (99.9%), calcium itaconate with one molecule of water of crystallization (99.3%), and magnesium itaconate with from 1 to 2 molecules of water of crystallization (107.6% based on 2 molecules of water of crystallization).

Itaconic acid is methylenesuccinic acid:



ACUTE EXPERIMENTS

All experiments were made in cats. Preliminary observations with intravenous doses of disodium itaconate showed that this material possesses a low order of toxicity, and that the factor of volume of fluid as well as tonicity might complicate the evidence of molecular toxicity. Accordingly, the concentration of disodium itaconate having the same tonicity as 0.85% solution of sodium chloride was determined. This was done by the red cell volume method. In preliminary tests with decreasing concentrations of disodium itaconate, hemolysis of the cat's red blood cells began to appear at a concentration of 1.4%. The cell volume method was then applied to solutions varying between 1.5% and 2.5%. Oxalated cat's blood was added in amounts of 0.5 or 1 cc. to each of several special graduated tubes containing 5 or 10 cc. of 0.85% solution of sodium chloride, or varying concentrations of disodium itaconate. They were allowed to stand for one hour and then centrifuged for one hour. The tube containing the 1.71% solution of disodium itaconate was found to have the same hematocrit value as the 0.85% solution of sodium chloride, hence, isotonic with cat's blood.

On the basis of the foregoing values, it is probable that the dissociation of the disodium itaconate in isotonic solution is practically complete. The 0.85% sodium chloride solution is an approximately 0.145 molar solution, and the 1.71% solution of disodium itaconate is an approximately 0.098 molar solution. Inasmuch as the sodium chloride solution is virtually completely dissociated, and the disodium itaconate solution is equitonic with it, it may be assumed that the dissociation of the latter is also virtually complete. There are, therefore, in equal quantities of isotonic solutions of the two materials, approximately 15 ions of sodium in sodium chloride for every 20 ions of sodium in disodium itaconate, or the disodium itaconate represents approximately 33% more sodium ions than does sodium chloride.

Effect of Intravenous (Single Dose) Disodium Itaconate.—The compound was injected in from 5% to 20% solutions intravenously in each of 17 cats in single doses of from 100 to 1500 mg. per Kg.

* Received Oct. 15, 1946, from the Department of Pharmacology, Cornell University Medical College, New York City.
† This study was supported in part by a grant from Chas. Pfizer and Co., Inc.

[1] The chemicals and assay values were supplied by Chas. Pfizer & Co., Inc. The high value for the magnesium salt was due to loss of water of crystallization during drying.

The injections were made at the approximate rate of from 1 to 4 cc. per minute. The volumes varied from 1 to 10 cc. per Kg. The *p*H of the solution was 7 6, determined by the glass electrode (Leeds-Northrop). The results are summarized in Table I.

In one group of experiments the volume of solution and the tonicity were the same for both materials. Each of 10 cats received an intravenous infusion of 117 cc. of an isotonic solution per Kg. at the rate of 0.5 cc. per Kg. per minute. In 4 of these cats, it was a solution of sodium chloride and in the remaining 6, disodium itaconate. The infusion was terminated after 117 cc. per Kg. were administered because such volumes, injected over a period of several hours, produced respiratory distress, and represent approximately tripling of the blood volume of the animal. The infusions were made from a burette into the femoral vein, without general anesthesia. When the infusion was ended the animal was released. There was some weakness in all cases. Those which received sodium chloride recovered completely without other signs of toxicity. Those which received disodium itaconate vomited, and 3 of the 6 cats died in convulsions in eight to thirty-six hours. In these experiments the dose of sodium chloride was 1 Gm. per Kg., and of disodium itaconate 2 Gm. per Kg., but on a molecular basis, the dose of sodium chloride was about 30% larger than that of disodium itaconate. These results, therefore, indicate that the molecular toxicity of disodium itaconate is greater than that of sodium chloride.

TABLE I.—EFFECT OF INTRAVENOUS DISODIUM ITACONATE IN CATS

Cat No.	Concentration, %	Dose (Mg./Kg.)	Effects	Recovery or Death
1	5	100	None	R
2	5	100	None	R
3	10	100	Depression	R
4	10	100	Depression	R
5	13.6	100	Vomiting, depression	R
6	13.6	100	Vomiting, depression	R
7	20.4	150	Vomiting, depression	R
8	20.4	150	Depression	R
9	20.4	150	Vomiting, depression	R
10	13.6	200	Vomiting, depression	R
11	5	250	Depression	R
12	10	250	Depression	R
13[a]	10	500	Depression, ataxia, tremors	R
14	10	500	Vomiting, depression	R
15	10	1000	Vomiting, depression	R
16[b]	15	1000	Vomiting, depression	R
17[c]	15	1500	Vomiting, depression	R
18	20.4	1500	Vomiting, depression	R
19	20.4	1500	Depression, ataxia, Convulsions	D
20	20.4	1500	Depression, ataxia, Convulsions	D

[a] This cat recovered and was employed again as No. 14 after 48 hours.
[b] This cat recovered and was employed again as No. 17 after 5 days.
[c] This cat recovered and was employed again as No. 18 after 48 hours.

These solutions of disodium itaconate were toxic to cats, causing depression, prostration, ataxia, vomiting, defecation, convulsions, increased respiration, accelerated heart rate, and vasodepression (see blood pressure experiments below). A dose of 100 mg. per Kg. was without appreciable effect as a 5% solution, it caused depression in the form of a 10% solution, and vomiting with depression when given as a 13.6% solution. This suggests hypertonicity as a possible cause of the toxic effects. The larger doses caused more severe symptoms with depression and prostration, and in fatal cases, convulsions. One animal survived a dose of 1.5 Gm. per Kg., and a similar dose repeated forty-eight hours later, while in others this dose proved fatal. The course of the toxic effects was brief. Vomiting or depression usually appeared within a few minutes, although in one case vomiting was first seen two hours after the injection. Recovery seemed complete in most cases within a period of three to eight hours, always within twenty-four hours. In the two fatal cases (1.5 Gm. per Kg.), death occurred in seventy-four minutes and ten hours, respectively.

In view of the fact that these severe toxic symptoms occurred after hypertonic solutions, from 3 to 12 times the isotonic, experiments were designed to compare the toxicity of solutions of sodium chloride with solutions of disodium itaconate in which tonicity and fluid volume were controlled.

This was confirmed by the next group of experiments in which the blood pressure was measured. The pressure was recorded from the carotid artery with a mercury manometer, the vessel being exposed with local anesthesia. In these experiments the effects of equi-molecular doses of sodium chloride and of disodium itaconate were compared.

TABLE II.—COMPARISON OF EQUI-MOLECULAR AND EQUI-TONIC SOLUTIONS OF DISODIUM ITACONATE AND SODIUM CHLORIDE ON BLOOD PRESSURE OF CATS[a]

Cat No.	Dose (Cc. per Kg.)	Blood Pressure: Control (Mm. Hg)	Blood Pressure: Change (Mm. Hg)	Remarks
		Disodium Itaconate		
1	8.77	120	−40	
	8.77	120	−60	Died 1 minute after injection
2	8.77	146	−106	Died 5 minutes after injection
3	8.77	170	−80	
	8.77	154	−154	Died during injection
4	8.77	140	−58	
	8.77	120	−120	Died during injection
5	8.77	150	−106	
	8.77	100	−70	Died at end of injection
		Sodium Chloride		
6	5.87	140	−30	
	5.87	132	−42	
	11.74	118	−68	Died approximately 12 hours after injection
7	5.87	156	−20	
	5.87	170	−34	
	11.74	130	−20	Died approximately 12 hours after injection
8	5.87	165	−35	
	5.87	150	−10	
	11.74	155	−49	Died 24 hours after injection
9	5.87	160	−24	
	5.87	134	−16	
	11.74	136	−18	Died 21 minutes after injection

[a] The concentrations of the solutions of disodium itaconate (17.1%) and sodium chloride (8.5%) were 10 times the isotonic. The intervals between the first and second doses were 5 minutes, and between the second and third doses, 15 minutes.

The injections were made in equi-tonic solutions, 10 times the isotonic, in order to keep the volume of solution low. For this purpose, each of 4 cats received a solution of 8.5% sodium chloride intravenously in 3 doses. The first dose was 5.87 cc. per Kg. (0.5 Gm. per Kg.) injected slowly into the femoral vein over a period of five minutes. This was repeated after an interval of five minutes. Double the dose was then given after an interval of fifteen minutes. In the case of the 5 cats receiving the disodium itaconate, a 17.1% solution was used, and the same procedure was followed except that the molecular dose equivalent to the 5.87 cc. of sodium chloride was 8.77 cc. (1.5 Gm. per Kg.) of the itaconate solution. The results are summarized in Table II. All animals died but it is clear that equi-molecular doses of disodium itaconate are much more toxic than sodium chloride, for in the case of the former all animals died either after the first or second dose, whereas in the case of the latter the first and second doses did not prove fatal. This is also borne out by the greater vasodepressor effect of the disodium itaconate.

Whether the greater toxicity of disodium itaconate is due to the entire molecule, the base, or the itaconate ion remains unsettled. There is indication that the free base may be the decisive factor in the difference in the toxicity of sodium chloride and disodium itaconate. In the foregoing comparisons of isotonic solutions in which the disodium itaconate was more toxic, the dose of the disodium itaconate represented about 33% more sodium ions than in the dose of sodium chloride.

TABLE III.—EFFECT OF DISODIUM ITACONATE AND SODIUM CHLORIDE ON THE CO_2-COMBINING POWER OF SERUM IN CATS[a]

Cat No.	Before (Vol. %)	After (Vol. %)	Change (Vol. %)
	Disodium Itaconate		
1	30.9	39.6	+8.7
2	41.3	50.1	+8.8
3	49.4	55.2	+5.8
4	41.6	49.3	+7.7
	Sodium Chloride		
5	42.5	40.6	−1.9
6	40.6	42.6	+2.0
7	39.5	43.2	+3.7
8	40.6	38.1	−2.5

[a] The solutions of disodium itaconate (1.71%) and sodium chloride. (0.85%) were isotonic with cat's blood. The gram-doses of the itaconate were larger than those of sodium chloride, but molecular-doses of itaconate were smaller.

That disodium itaconate tends to produce an alkalosis is shown by the results summarized in Table III. The CO_2-combining power of the venous blood serum was made before, and at the end of the infusion in the foregoing experiments. In the case of the sodium chloride, 2 of the cats showed an average fall of 2.2 volumes per cent, and 2 an average rise of 2.8 volumes per cent, while in the case of the disodium itaconate, there was an average rise of 7.75 volumes per cent.

Effect of Intravenous Magnesium Itaconate.—This was tested in each of 4 cats. The drug was given in 5% and 10% solutions. The injections were given at an approximate rate of 4 cc. per Kg. One animal died during the injection with a dose of 500 mg. per Kg., another after 250 mg. per Kg.,

and a third within one minute after 150 mg. per Kg. The fourth animal received doses of 50 mg. per Kg. at intervals of fifteen minutes. The first injection caused no effect, the second produced some depression, and the last (total 400 mg. per Kg.) proved fatal. Death resulted from respiratory paralysis. Magnesium itaconate, therefore, appears to be much more toxic than disodium itaconate, and these experiments indicate that it is about 10 times as toxic as the disodium salt.

Effect of Disodium Itaconate on Blood Pressure.—In the previous section it was pointed out that massive doses of disodium itaconate (1.5 Gm. per Kg.) injected intravenously caused abrupt fall of the blood pressure with fairly rapid recovery (five to fifteen minutes). The effect of smaller doses was tested in each of 7 cats. The drug was injected intravenously in concentrations of from 5% to 10% (approximately 3 to 6 times isotonic) at the rate of about 4 cc. per minute. The animals were anesthetized with pentobarbital-sodium (30 mg. per Kg. intraperitoneally), and the blood pressure was recorded from the carotid artery by means of a membrane manometer. Respiration was recorded from an intrapleural cannula. The doses ranged from 100 to 700 mg. per Kg. Doses of 100 mg. produced no appreciable effect, those of 200 mg. or more produced sharp declines of the blood pressure of 20 to 60 mm. Hg, with recovery in one or two minutes. The extent of the blood pressure fall increased with the dose, and doses as high as 500 mg. lowered the pressure to shock levels, but, even in these, recovery was complete within fifteen minutes. The effect on the blood pressure may be due to either the hypertonicity of the solution or the high concentration of the material in the blood stream, for in one cat which received 100 mg. per Kg. at intervals of ten minutes, there was no significant effect on the blood pressure even after a total of 600 mg. per Kg. A fleeting increase in the respiration occurred with all doses, even those which produced no change in the blood pressure.

Effect of Disodium Itaconate on the Electrocardiogram.—One cat received an intravenous injection of a 10% solution at a rate of 4 cc. per minute. An electrocardiogram (Lead II) was taken before a dose of 200 mg. per Kg., and at intervals of about two minutes during a period of eleven minutes after the injection. There were no significant changes. The second animal was anesthetized with pentobarbital-sodium (30 mg. per Kg. intraperitoneally), and the blood pressure was recorded from the carotid artery. It received a total of 1.5 Gm. of disodium itaconate per Kg. in a 10% solution in fractions of 100, 200, 500, and 700 mg. per Kg., respectively, at intervals of approximately ten minutes. The total volume of fluid was 15 cc. per Kg. The blood pressure declined from 150 to 95 at the end of the last injection. The electrocardiogram showed a marked change; high positive T-waves in Lead II before the injection, became lower and then negative after the 100-mg. dose, and then more deeply negative after the subsequent injections. Whether some of these changes are caused by the action of the drug, by hypertonicity of the solution, or by the volume of fluid giving rise to ventricular strain, remains unsettled.

Effect of Oral (Single Dose) Itaconates.—Itaconic acid, calcium itaconate, magnesium itaconate, and disodium itaconate were administered orally in 54 cats. The results are summarized in Table IV. They were given by stomach tube in varying concentrations, or in the form of capsules. The doses varied from 100 mg. to 5 Gm. per Kg. The oral doses of all 4 compounds caused vomiting and/or diarrhea, in doses of 500 mg. per Kg. or larger. The more concentrated solutions were more apt to cause vomiting as shown in the case of the disodium itaconate; 4 of 5 vomited after the 500-mg. dose when given as a 5% solution, whereas none of the 5 vomited when the same dose was given as a 1.71% solution which is isotonic.

Doses up to 1 Gm. per Kg. by oral administration failed to show signs of systemic toxicity.

In view of the tendency to vomiting by what appears to be a local action, attempts were made to determine absorption and systemic toxicity by preventing vomiting. Each of 4 cats received morphine prior to the oral itaconate, but 3 of these vomited and 3 developed diarrhea. In these, the 3 which received 5 Gm. per Kg. developed convulsions and died in from 2 to 9 hours; the one with 4 Gm. per Kg. survived, probably due to the loss of the drug through vomiting. In 2 additional animals the loss of the drug through vomiting was prevented by placing a ligature above and below the stomach and introducing the material directly into the lumen of the stomach; in these a dose of 5 Gm. per Kg. caused convulsions and death. In one animal a dose of 5 Gm. per Kg. was administered by stomach tube and all the vomitus was returned by stomach tube; this proved fatal.

The foregoing results show that the oral toxicity of itaconic acid and its salts is very low, and also that absorption occurs, for in massive doses the disodium itaconate produced death in convulsions. The response of the cat to such doses of disodium itaconate is similar to that after similar doses of sodium bicarbonate or sodium succinate (3).

CHRONIC EXPERIMENTS

Chronic experiments were carried out with each of 6 cats, 2 of which received itaconic acid orally, 2 disodium itaconate orally, and 2 disodium itaconate intravenously. The dose of itaconic acid was 100 mg. per Kg. daily (6 days a week), and the salts were given in approximately equivalent doses, 150 mg. per Kg. The oral doses were administered in the form of a capsule, and the intravenous doses in a 5% solution injected into the femoral vein. The treatment period lasted fourteen weeks. The animals were kept on a constant daily diet of 12.5 cc. of evaporated milk (diluted to 125 cc.) and 150 to

TABLE IV.—EFFECT OF ITACONIC ACID, CALCIUM, MAGNESIUM AND DISODIUM ITACONATE BY ORAL ADMINISTRATION IN CATS

No. of Cats	Concentration	Dose (Mg./Kg.)	Effect
		Itaconic acid	
4	2 in capsules; others 5%	100	None
5	2 in capsules; others 5%	250	None in 1; depression in 2; vomiting and depression in 2
1	5%	500	Vomiting
		Calcium Itaconate	
2	5% (suspension)	250	None
2	5% (suspension)	500	Vomiting in 1; diarrhea in 1
2	Capsules	1000	Vomiting
		Magnesium Itaconate	
2	5%	250	None
2	5%	500	Vomiting in 1; diarrhea in 1
		Disodium Itaconate	
2	Capsules	100	None
9	2 in capsules; 2 in 5%; others 17.1%	250	None
15	5 in capsules; 5 in 5%; others 1.71%	500	No effect in 6; vomiting in 9
1	5%	1000	Vomiting
1	17.1%	4000	Vomiting; depression for 48 hours[a]
3	17.1%	5000	Vomiting in 2; diarrhea, depression, ataxia, prostration, convulsions, death with respiratory paralysis in 2, 2.5, and 9 hours, respectively[a]
2	17.1%	5000	Retching, depression, ataxia, prostration, convulsions in 3 hours, death with respiratory paralysis in 4 hours[b]
1	17.1%	5000	Vomiting, diarrhea, depression, convulsions, death in approximately 15 hours[c]

[a] Cats received 1 mg./Kg., of a solution of 1% morphine sulfate intravenously 6 minutes before the itaconate.
[b] The stomach was exposed and a ligature placed at the lower end of the esophagus and at the jejunum in one cat, and at the lower end of the esophagus and at the iliocolic junction in the second cat. The drug was injected into the lumen of the stomach. Ether anesthesia was used.
[c] The dose was administered in 5 equal parts at 30-minute intervals and all vomitus was readministered by stomach tube.

200 Gm. of beef lung, and once a week 150 Gm. of beef spleen.

Observations were made on the general behavior of the animals, their weight; liver function with the bromsulphalein test; urine for albumin, sugar, casts, cells, *p*H, and specific gravity; blood NPN and creatinine; kidney function by the PSP test; red and white blood cell count; hemoglobin; and blood CO_2-combining power. Values for the foregoing were obtained during a control period of five weeks prior to the administration of the drugs. In 2 additional cats which received no drug, similar examinations were made for control purposes throughout the period of nineteen weeks. The samples for blood tests were taken from the femoral vein with the animal in a fasting state, except for the blood counts and hemoglobin for which samples were taken from the ear vein.

The general appearance and behavior of the animals showed no change during the course of the chronic experiments. There was no vomiting or diarrhea. The average weight during the control period was 3.52 Kg. and during the treatment period 3.46 Kg.

There were 118 specimens of urine which showed an average *p*H of 6.2 during the control period and 6.1 during the treatment period. The specific gravity average 1.029 during the control and 1.021 during the treatment period. The examinations for casts, albumin, sugar, and blood cells in the urine showed no significant abnormalities.

Phenolsulfonphthalein Test.—This test was carried out 33 times. A dose of 1 cc. containing 6 mg. of the dye was injected intravenously after the bladder was emptied by expression. The urines, similarly obtained, were tested at the end of one and again in two hours after the dose. The total percentage excretion in two hours averaged 50.3 for the 16 control tests, and 57.3 for the 17 tests during the treatment period, the last test being made at the end of the treatment period.

Bromsulphalein Test.—The method of Rosenthal and White (4) was used. The 2-mg. dose of the dye was injected intravenously and the blood was tested after thirty minutes. There were in all 34 tests, 19 in the control period and 15 during the treatment period. All specimens were negative for dye in thirty minutes after the injection.

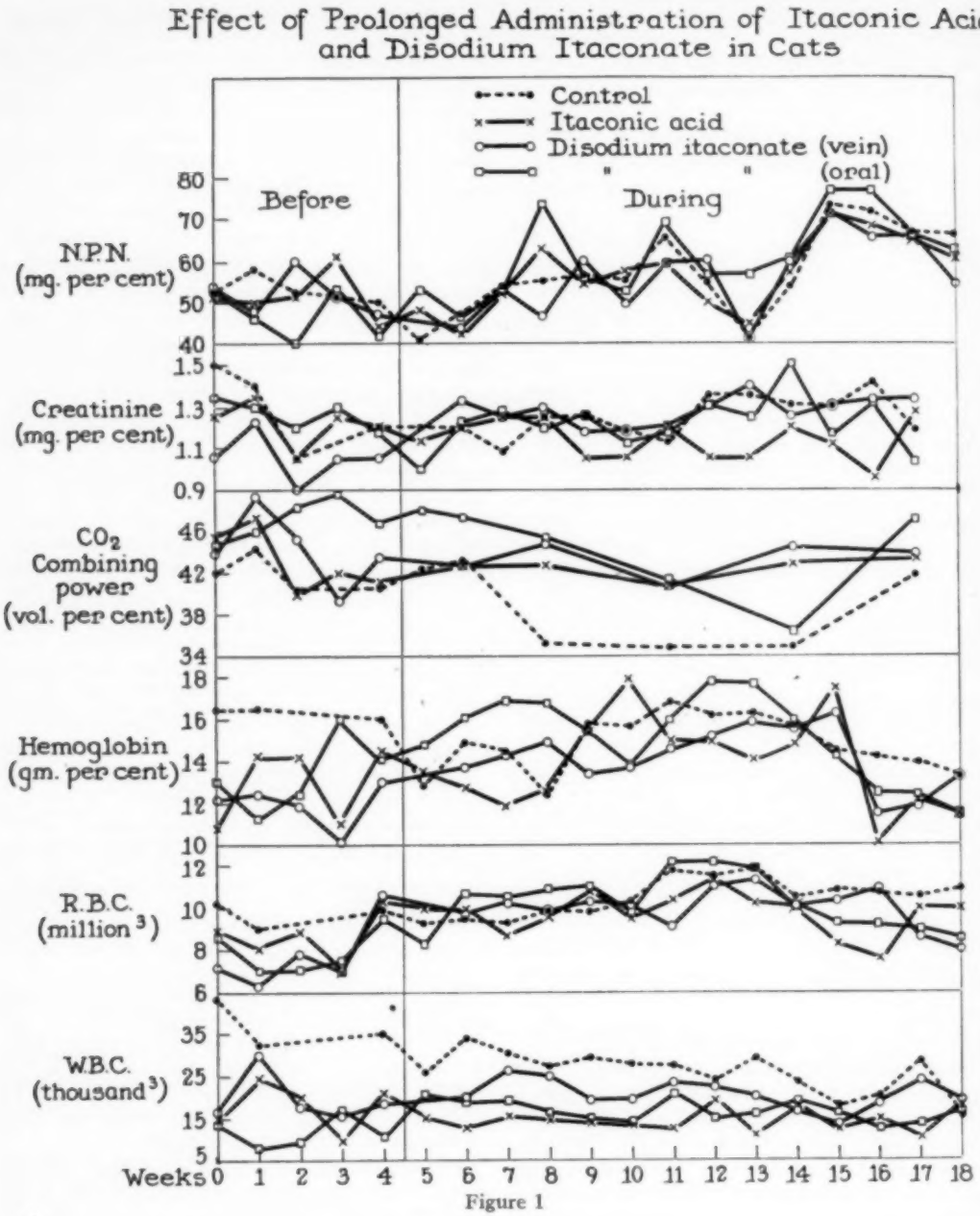

Effect of Prolonged Administration of Itaconic Acid and Disodium Itaconate in Cats
Control
Itaconic acid
Disodium itaconate (vein)
" (oral)
Before
During
N.P.N. (mg. per cent)
Creatinine (mg. per cent)
CO_2 Combining power (vol. per cent)
Hemoglobin (gm. per cent)
R.B.C. (million^3)
W.B.C. (thousand^3)
Weeks 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 1

The results of the remaining blood tests are summarized in Fig. 1. It may be noted that the protracted use of the itaconates in doses equivalent to 100 mg. of itaconic acid per Kg. produced no significant change in the blood NPN, creatinine, red and white blood cells, hemoglobin, and CO_2-combining power.

Electrocardiogram.—In the case of each of the 2 cats which received the intravenous doses of disodium itaconate, an electrocardiogram was taken before the treatment was started, and again at the end of the fourteen weeks of treatment. Both tracings were normal and showed no changes.

Histological Examination of Liver and Kidneys.—The post-mortem examination of all 6 cats treated with the itaconates for protracted periods showed no conspicuous abnormalities in the liver, kidneys, spleen, lungs, heart, adrenals, and gastrointestinal tract. A section of the liver and kidney from the animals in each type of experiment was fixed in formalin and stained. The histological examination of the 16 sections showed no abnormalities.

A more severe test of toxicity was carried out in one cat which received a total of 12.2 Gm. of disodium itaconate per Kg. intravenously in doses varying from 500 to 1100 mg. per Kg. at intervals

of from two to seven days during a period of six weeks. All but the first dose caused vomiting within one to five minutes, lasting during a period of from one to five minutes. The animal weighed 3.2 Kg. at the beginning of the experiment and 3 Kg. at the end. After the last dose the animal was sacrificed. Post-mortem examination showed no gross abnormalities. The histological examination of the liver and kidneys of this animal was essentially negative.

SUMMARY

The present report deals with the effects of itaconic acid, and its disodium, calcium, and magnesium salts in cats.

Observations were made after single intravenous and oral doses, and during the use of daily intravenous and oral doses for a period of fourteen weeks. The study includes the effects on the general behavior of the animal, the state of nutrition, the heart, the blood pressure, gastrointestinal tract, liver function, kidney function, and blood.

The most detailed study was made in the case of the disodium salt of itaconic acid. The results show that this salt gives rise to vomiting and/or diarrhea after doses of the order of about 0.5 Gm. per Kg. or larger, apparently due to local action in the gastrointestinal tract, but without signs of systemic poisoning. Oral doses of 5 Gm. per Kg. proved fatal after severe gastrointestinal disturbances, prostration, and convulsions. The effects are similar to those of similar doses of sodium succinate and sodium bicarbonate. By intravenous injection smaller doses proved fatal, 1.5 Gm. per Kg., and one-tenth as much given intravenously caused vomiting and depression. The intravenous doses produced an abrupt fall of the blood pressure with prompt recovery in nonfatal doses, also inversion of the T-wave of the electrocardiogram.

As was observed in the case of sodium succinate, a dose of morphine which is effective in suppressing the emetic action of several other drugs, did not prevent vomiting or diarrhea by disodium itaconate.

A limited number of experiments with itaconic acid, and the calcium and magnesium salts indicate that these produce gastrointestinal symptoms after oral administration, similar to those of the disodium salt. Their oral toxicity is low, since doses of 0.5 and 1 Gm. per Kg. failed to cause systemic poisoning. The magnesium salt appeared to be about 10 times as toxic as the disodium salt by intravenous injection.

The protracted daily administration of nontoxic doses of itaconic acid, 100 mg. per Kg. orally, and equivalent doses in the form of the disodium salt orally and intravenously, for periods of many weeks failed to produce poisoning as indicated by the state of nutrition, the blood, liver function, kidney function, the electrocardiogram, and histological examination of the liver and kidney.

A comparison of the toxicity of disodium itaconate with sodium chloride in experiments in which the tonicity of the solution and volume of injected fluid were controlled, indicates that the disodium itaconate has a higher molecular toxicity than sodium chloride. Whether this is due to the entire molecule, the itaconate ion, or the base remains uncertain. There is indication that the itaconate ion is substantially inert, and that the systemic effects produced by the massive doses, aside from the effects of excessive volume of fluid and hypertonicity, is the result of the base content of the salt.

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Influence of Time of Application of Fertilizers on Growth and Alkaloidal Content of *Hyoscyamus Niger* Linn.*

By S. PRASAD†

The influence of time of application of fertilizers containing nitrogen, phosphorus, and potassium to *Hyoscyamus niger* Linn. is reported. The effect of varying the dose and the number of applications of fertilizer and their influence on the alkaloidal content of the treated plants of *Hyoscyamus niger* L. was determined. Data used were statistically analyzed to test the significance of the results.

IN A PREVIOUS contribution (1), the effect of nitrogen, phosphoric acid, and potash, on the growth and alkaloidal content of *Hyoscyamus niger* Linn., was recorded. The results obtained stressed the importance of nitrogen and potassium in the production of greater growth and alkaloidal content in these plants. No light has so far been thrown on the critical period of nutrient requirement. The amount of manurial ingredients necessary for the optimum growth of plants, at different stages of their growth, is a factor of great importance from the standpoint of yield and elaboration of organic substances. The present investigation is therefore devoted to the study of the effects produced in these directions, by the application of nutrients in varying combinations and ratios at one or different stages of the growth cycle of *Hyoscyamus niger* L.

MATERIALS AND METHODS

Healthy seeds of *Hyoscyamus niger* L. were selected and germinated in well-prepared seed-beds. Seedlings of 2–3 cm. in length were carefully uprooted and transplanted in the experimental plots. The preparations of these plots for sowing was done in the manner described elsewhere (1).

Plan and Layout of the Experiment

System of Replication.—Four randomised blocks of 14 treatments each. The plots were manured with ammonium sulfate (20.6% N), 600 Gm. per plot; bone meal (21.0% P_2O_5), 300 Gm. per plot and potassium sulfate (48.0% K_2O), 100 Gm. per plot. The plants were supplied with different amounts of these manures at 3 stages namely (*a*) ten days, (*b*) forty days, and (*c*) seventy days, after transplantation, according to the schedule of manuring given in Table I. In all cases the total amount of nutrients added remained the same; only the period of the life cycle when they were supplied varied from treatment to treatment.

Sampling of plants for measurement of growth characters and the procedure for determining the alkaloidal content were similar to those described previously (1).

EXPERIMENTAL FINDINGS

Plant Appearances.—Soon after the application of manures to the plants, marked differences in their development and growth became discernible. Plants that received nitrogen from the beginning (Treatment Nos. 1, 6, 8, 10, 12, and 13), either in full dose or in parts, showed a better and sturdier growth and presented a verdant general outlook as compared to those receiving only PK or being devoid of any manurial supply (Treatment Nos. 2, 3, 4, 5, 7, 9, 11, and 14). After the addition of the second dose of manure at the "B" stage (forty days after transplantation), plants that received one-half the dose of NPK at "A" stage (Treatment No. 10) proved to be better benefited as compared to even those that received full dose of manures from the beginning (Treatment No. 1). Such plants as received nitrogen at this stage (Treatment Nos. 2, 4, 7, 9, and 11) were able to make good the shortcoming in their growth to a great extent and afterward seemed to approach, in their development, those plants that received nitrogen from the very beginning. But the plants that were supplied with nitrogen at a very late stage, *viz.*, seventy days after transplantation (Treatment Nos. 3 and 5) could not make good this lag in their growth and development to that extent as those that received nitrogen at the "B" stage. Plants which were manured at "C" stage started to develop bigger leaves and sturdier new branches and even the leaves that were already formed increased somewhat in size; the delay, however, was so great that they failed to reach the size of the plants that received nitrogen from the beginning. Treatment Nos. 11, 12, and 13 that were treated with nitrogenous manure previously either at "A" or at "B" stage and received again this manure at the "C" stage, were slightly less sturdy

* Received Nov. 27, 1946, from the Department of Pharmaceutics, Benares Hindu University, Benares, India.
† Present address: School of Pharmacy, University of Wisconsin, Madison, Wis.

in their development as compared to those receiving nitrogen from the beginning. Treatment No. 13 however, developed taller branches with bigger leaves than Treatment Nos. 11 or 12.

At the fifth month of their growth, when the plants had attained their full development, Treatment No. 10 which was provided with NPK in 2 installments—one-half at "A" and the remaining half at "B" stage—showed the best growth. Next in order of good growth were Treatment Nos. 1, 13, 6, 8, 9, 2, 12, 7, 11, and 4. Treatment Nos. 3 and 5 presented poorer growth but were better than the control.

Dry Matter Production.—Nitrogen likewise increased consistently the dry matter yield of the entire plant (Table II) in those cases where this ingredient was provided from the very beginning of their life (Treatment Nos. 1, 6, 8, 10, and 12). Treatment No. 13, however, did not respond significantly at the third month of growth; this was possibly due to the supply of the one-third of the normal dose proving insufficient to meet the entire demand at this period of active growth. Plants which received nitrogen at the "B" stage (Treatment Nos. 2, 4, 7, 9, and 11) responded significantly only from the

TABLE I.—SCHEDULE OF MANURING

Treatment No.	Particulars[a]	Manures in Gm./Plot Applied at: 10 Days after Transplantation N	P	K	40 Days after Transplantation N	P	K	70 Days after Transplantation N	P	K	Total N	P	K
1	$N_AP_AK_A$	600	300	100	...	...	...	...	...	...	600	300	100
2	$N_BP_BK_B$	..	...	...	600	300	100	...	...	...	600	300	100
3	$N_CP_CK_C$	..	...	...	...	...	...	600	300	100	600	300	100
4	$N_BP_AK_A$	...	300	100	600	...	...	...	...	...	600	300	100
5	$N_CP_AK_A$	...	300	100	...	...	...	600	...	...	600	300	100
6	$N_AP_BK_A$	600	...	100	...	300	...	...	...	...	600	300	100
7	$N_AP_CK_A$	600	...	100	...	...	...	...	300	...	600	300	100
8	$N_AP_AK_B$	600	300	...	...	...	100	...	...	...	600	300	100
9	$N_AP_AK_C$	600	300	...	..	...	...	...	...	100	600	300	100
10	$^1/_2(NPK)_A + ^1/_2(NPK)_B$	300	150	50	300	150	50	...	...	...	600	300	100
11	$^1/_2(NPK)_B + ^1/_2(NPK)_C$	...	...	...	300	150	50	300	150	50	600	300	100
12	$^1/_2(NPK)_A + ^1/_2(NPK)_C$	300	150	50	...	...	...	300	150	50	600	300	100
13	$^1/_3(NPK)_A + ^1/_3(NPK)_B + ^1/_3(NPK)_C$	200	100	$33^2/_3$	200	100	$33^1/_3$	200	100	$33^1/_3$	600	300	100
14	Control	...	...	...	...	...	...	...	...	...	...	...	...

[a] Letters A, B, and C below the fertilizers N, P, and K represent the stages of manurial supply, namely, 10 days, 40 days and 70 days, respectively, after the plants were transplanted to the plots.

Shoot Length.—The effect of application of nitrogen was clearly noticeable in the increase of height of these plants. Plants supplied with nitrogen along with P or K from the beginning (Treatment Nos. 6 and 8) were as good in their height as those that received the full combination NPK (Treatment Nos. 1, 10, 12, and 13). If this full dose of NPK was split up into 3 installments of one-third each, the height attained was no doubt less than if it was split up into one-half. In the latter case, if the supply of one-half dose was given at "A" and the other half at "B", the effect was much better on the plants than when the half doses were supplied either at "A" and "C" (Treatment No. 12) or at "B" and "C" (Treatment No. 11) stages.

Plants receiving nitrogenous manures from the beginning (Treatment Nos. 1, 6, 8, 10, 12, and 13) showed significant increases in height, consistently at all the stages in their life cycle; those that received this manurial ingredient at the "B" stage (Treatment Nos. 2, 4, 7, 9, and 11) showed significant increases only after twenty days of application of these manures. In the latter case some treatments like Nos. 11 and 9 did not show consistently significant effects at the different stages in the life cycle of plants (Table II). A delayed supply of nitrogen at the "C" stage (Treatment Nos. 3 and 5) did not enable the plants to respond significantly in this direction.

fourth month onward; they could not counterbalance the loss in dry matter accumulation earlier which they otherwise would have done if the manures were made available from the beginning. This is further borne out by the dry matter accumulation of the plants receiving nitrogen at the "C" stage (Treatment Nos. 3 and 5). These plants were not able to make up the shortcoming in their dry matter yield even at their fifth month of growth, because of the too-long delayed supply of this essential element.

On the dry weight of leaves, the responses of these manurial ingredients were more or less similar to that noted in the entire plant (Fig. 1). Treatment Nos. 1, 6, 8, 10, 12, and 13 showed significant effects consistently at all the stages, because of an early supply of nitrogenous manure. Treatment Nos. 2, 4, 7, 9, and 11, receiving nitrogen at "B" stage, on the contrary, exhibited significant increases from the fourth month onward, in majority of these treatments and at the third month in case of treatment No. 4 only. Treatment Nos. 3 and 5 did not show significant increases till the end, i.e., fifth month of their life cycle.

On dry matter accumulation of stem, the effect of these fertilizers was somewhat different from that noted on the entire plant or on the leaves. Early treatment with nitrogen did not bring about significant responses in these plants (1). This appears

TABLE II.—AVERAGE PLANT CHARACTERS BELONGING TO DIFFERENT TREATMENTS

Treatment No.	Age in Months 2	3	4	5
	Stem Length in Inches			
1	3.26[a]	10.90[a]	25.00[b]	27.88[b]
2	2.15	9.38[a]	22.50[b]	27.00[b]
3	2.20	6.73	20.83	22.13
4	2.00	9.20[a]	22.17[b]	26.88[b]
5	2.00	6.45	18.70	21.00
6	3.18[a]	10.05[a]	24.00[b]	26.50[b]
7	2.10	8.78[a]	22.80[b]	23.63
8	3.20[a]	10.17[a]	23.70[b]	26.00[b]
9	2.00	9.05[a]	22.90[b]	23.15
10	3.20[a]	10.97[a]	27.70[a]	30.88[a]
11	2.10	8.65[a]	19.83	22.13
12	3.20[a]	10.20[a]	22.17[b]	26.63[b]
13	3.10[a]	8.78[a]	22.60[b]	26.25[b]
14 (Control)	2.10	6.20	16.17	18.00
S.E.[c]	±0.211	±0.241	±3.108	±4.063
	Dry Weight of the Whole Plant in Gm.			
1	0.309[a]	1.646[b]	23.854[a]	51.551[a]
2	0.154	1.063	17.793[a]	37.866[a]
3	0.156	0.685	11.045	24.077
4	0.120	1.324	16.366[a]	35.303[a]
5	0.118	0.656	11.509	23.124
6	0.308[a]	1.171	21.628[a]	42.452[a]
7	0.174	1.013	15.293[a]	35.723[a]
8	0.274[b]	1.272	20.013[a]	41.330[a]
9	0.206	1.030	13.527[b]	38.879[a]
10	0.309[a]	1.650[b]	26.113[a]	54.054[a]
11	0.191	0.999	16.250[a]	35.324[a]
12	0.302[a]	1.433[b]	18.415[a]	37.658[a]
13	0.290[b]	1.082	18.007[a]	42.846[a]
14 (Control)	0.126	0.644	7.903	16.745
S.E.[c]	±0.057	±0.405	±2.210	±3.642
	Dry Weight of Leaf in Gm.			
1	0.201[a]	1.159[a]	15.845[a]	26.325[a]
2	0.087	0.656	10.946[a]	20.031
3	0.087	0.329	6.084	11.103[a]
4	0.056	0.926[b]	9.863[b]	17.368
5	0.056	0.216	7.460	11.420[a]
6	0.201[a]	0.749[b]	14.243[a]	21.856[a]
7	0.068	0.706	10.104[a]	18.625[a]
8	0.162[a]	0.917[b]	13.025[a]	20.920[a]
9	0.098	0.700	8.563[b]	18.756[a]
10	0.190[a]	1.210[a]	17.627[a]	28.382[a]
11	0.125[b]	0.662	10.380[b]	16.346
12	0.190[a]	1.065[a]	11.592[a]	16.826[a]
13	0.185[a]	0.758[b]	11.468[a]	22.625[a]
14 (Control)	0.064	0.358	4.480	8.968
S.E.[c]	±0.028	±0.212	±2.104	±2.320
	Dry Weight of Stem in Gm.			
1	0.078	0.325[b]	6.985[a]	22.205[a]
2	0.045	0.304[b]	5.863[b]	15.389
3	0.046	0.282	4.225	11.012
4	0.034	0.290	5.658[b]	15.923[a]
5	0.038	0.202	3.243	10.012
6	0.075	0.276	6.453[b]	18.286[a]
7	0.082[b]	0.213	4.386	15.025[a]
8	0.078	0.240	6.046[b]	18.321[a]
9	0.083[b]	0.210	4.200	17.021[a]
10	0.085[b]	0.298[b]	7.243[a]	22.216[a]
11	0.042	0.214	5.005	16.986[a]
12	0.082[b]	0.240	5.843[b]	18.302[a]
13	0.074	0.208	5.650[b]	18.205[a]
14 (Control)	0.040	0.200	3.002	6.217
S.E.[c]	±0.020	±0.048	±1.201	±2.439

(*Cont'd*)

to be most probably due to the fact that in *Hyoscyamus niger* L. the plant forms good rosettes of leaves in the early stages and stem elongation takes place later in the third month of the life cycle. Hence, if the essential manurial ingredients like nitrogen are present from the very beginning, good rosettes of large leaves are formed first and later on stem and branches are formed. If, however, nitrogen is poorly supplied, the plants show a tendency to increase in length first, bearing alternate leaves of smaller size. At a later stage, when the nitrogen is present and when leaves have formed, increase in dry matter accumulation of stem takes place. This has been pointed out (1) and is borne out by the results obtained in this experiment. The delayed supply of nitrogen did not lead to significant effects in the case of stem dry weight (Treatment Nos. 3 and 5). The presence of P and K, without nitrogen, either from the beginning or during the later stages showed no effect on the production of dry matter.

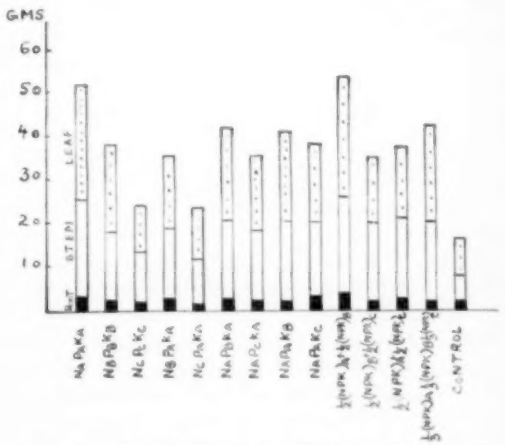

Fig. 1.—Final dry weight of the entire plant or its component organs in the different treatments. Letters A, B, and C below the fertilizers N, P, and K represent the stages of manurial supply, namely, 10, 40, and 70 days, respectively, after the plants were transplanted to the plots.

TABLE II (*Cont'd*)

Treatment No.	Age in Months 2	3	4	5
	Dry Weight of Root in Gm.			
1	0.030	0.162[a]	1.024[a]	3.021[a]
2	0.022	0.102	0.984[a]	2.456[a]
3	0.023	0.074	0.736	1.962[b]
4	0.030	0.108[b]	0.845[b]	2.012[a]
5	0.024	0.138[a]	0.806[b]	1.692
6	0.032	0.146[b]	0.932[b]	2.310[b]
7	0.024	0.094	0.803[b]	2.015[b]
8	0.034	0.115[b]	0.942[b]	2.089[b]
9	0.025	0.120[a]	0.764	3.102[a]
10	0.034	0.152[a]	1.243[a]	3.456[a]
11	0.024	0.123[a]	0.856[b]	2.082[b]
12	0.030	0.128[a]	0.980[a]	2.530[a]
13	0.031	0.126[a]	0.889[b]	2.016[b]
14 (Control)	0.022	0.086	0.421	1.560
S.E.[c]	±0.008	±0.010	±0.185	±0.210

[a] Significant at 1% (compared with control).
[b] Significant at 5% (compared with control).
[c] Standard error of difference of means.

On the dry matter production in roots, the effect of nitrogen is as important as that of P and K, inasmuch as the plants belonging to Treatment No. 5, which were supplied with PK at an early stage, did show significant effects even at the third month when no nitrogen was available. Nitrogen, however, has marked effect in the production of root substance, in that the plants supplied with this manurial ingredient showed always a higher value than those devoid of it (Table II).

On alkaloid formation, too, nitrogen produced significant effects; plants receiving this element from the very beginning of their life cycle showed a greater alkaloidal content than those which were supplied with this element at a later stage. The highest alkaloidal content was obtained in Treatment No. 10; next in order were Treatment Nos. 1, 6, 13, and 7 (Fig. 2). In these the alkaloidal content of the leaves significantly increased over the control. In others, the increases were not significant at all. (Table III.)

TABLE III.—PERCENTAGE OF ALKLAOIDS IN VARIOUS TREATMENTS[a]

Treatment No.	Percentage of Alkaloids	Treatment No.	Percentage of Alkaloids
1	0.0660[b]	8	0.054
2	0.0592	9	0.0540
3	0.0570	10	0.0692[c]
4	0.0596	11	0.0615
5	0.0560	12	0.0600
6	0.0654[b]	13	0.0650[b]
7	0.0648[b]	14 (Control)	0.0560

S. E. of difference of means ± 0.0004

[a] The plants were analyzed for the alkaloidal content when they were four months old.
[b] Significant at 5% (compared with control).
[c] Significant at 1% (compared with control).

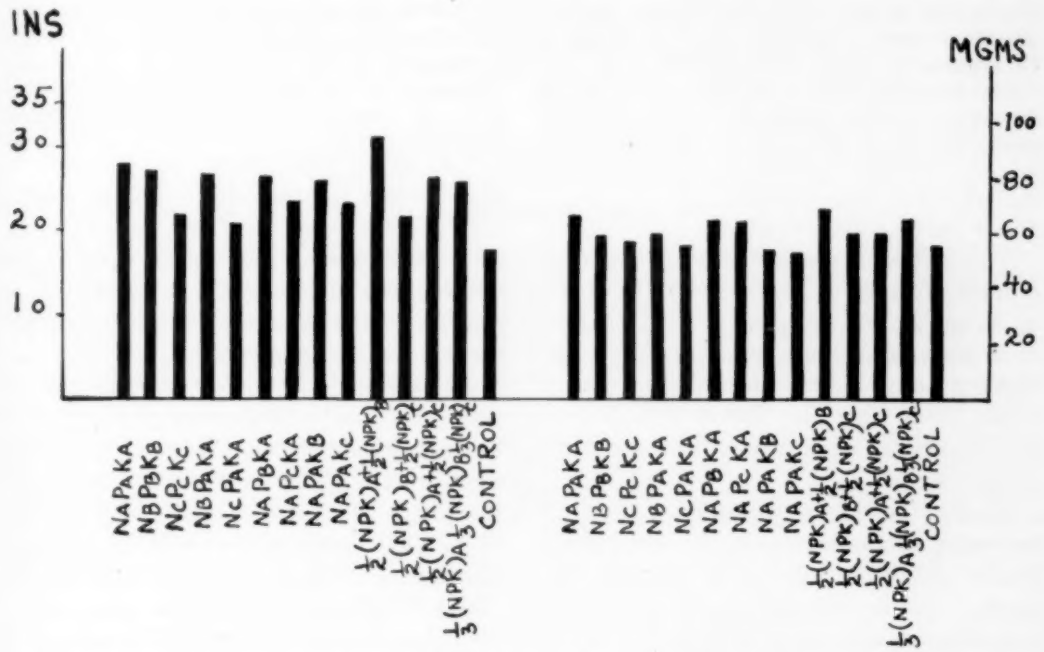

Fig. 2.—Final measurements of stem height and alkaloidal content in the different treatments. The ordinate on the left side represents stem height in inches and that on the right, alkaloidal content in milligrams. Letters A, B, and C below the fertilizers N, P, and K represent the stages of manurial supply, namely, 10, 40, and 70 days, respectively, after the plants were transplanted to the plots.

DISCUSSION

It will be seen from the results narrated above that, in so far as general growth, stem elongation, and dry matter production are concerned, the effect of nitrogen is very pronounced. An early supply of this element leads to an increase of these growth characters, while a delayed supply brings about a poor and stunted growth and leads to reduction in dry matter and alkaloid formation. If the supply of this essential element is withheld for a very long time—until the period of active vegetative growth comes to an end—then it is not possible to counterbalance the harmful effects of nitrogen-starvation in these plants even when the supply is made good at later period. Of course, on the stem, the effect of nitrogen deficiency is not so marked as on the leaves, particularly during the early stages. The reasons for this differential behavior have already been explained. In the end the effect of a long starvation of nitrogen is also noticeable in the decreased production of stem dry matter, due apparently to the

poor development of branches or stalk bearing leaves. With nitrogen present from the very beginning, the formation of good rosettes of large leaves takes place abundantly, followed by the production of long branches with leaves, both of which contribute significantly in increasing the vigor of the plants.

If the complete dose of the manurial ingredients (N, P, and K) is split into halves or thirds, and these part doses are supplied at different stages, the effects are equally characteristic; it is to be seen that splitting up into one-third dose does not lead to so beneficial results as splitting into one-half. In the latter case, if the half-doses are applied one at the beginning and the other during the period of active growth, there is an all-round better effect on the growth and alkaloid formation in these plants. This fact has been borne out by the behavior of plants belonging to Treatment No. 10, as compared to those of Treatment Nos. 11, 12, and 13. In view of the high requirements of nutrients, during the middle stage of life cycle, their adequate supply is absolutely necessary to meet the requirements of the growing organisms. This probably is not satisfied in Treatment No. 13 where only one-third the quantity is supplied during this period. In treatment Nos. 10, 11, and 12 where 50% of the total quantity is available, the plants show better performance of growth and higher alkaloid formation. Further, one-third the total quantity might well suffice to yield optimum results, if applied when the plants are young, but the same quantity fails to bring about similar effects if applied at a later stage of active differentiation. Phasic requirement of the plant varies remarkably; it is usually high during middle periods, between the second and third months of the life cycle, when an adequate supply of these manurial ingredients is found conducive to high vigor and greater alkaloid formation. While nitrogen is required from the early stages, the other two ingredients, namely, phosphorus from bone meal and potassium sulfate, are needed most at about seventy days after germination of these plants. It is suggested that for securing maximum benefit these ingredients may well be applied in suitable doses at these critical stages.

For alkaloid formation, too, nitrogen and potassium have the most marked effect and that, too, when they are applied at forty and seventy days after germination, as will be testified from the fact that Treatment No. 10, which received these ingredients at the above-mentioned stages, possessed the highest alkaloidal content.

SUMMARY

The present investigation deals with the influence of time of application of fertilizers N, P, and K, when applied in complete dose or in parts, at 3 stages, *viz.*, ten, forty, and seventy days after transplantation of *Hyoscyamus niger* L. The data have been statistically analyzed to test the significance of the result.

Plants receiving nitrogen from the very beginning of the life cycle, show decidedly greater growth of leaves and branches and produce sturdier plants than those receiving this essential ingredient at later stages.

If the supply of nitrogen is withheld for a long time, till the plant completes its active growth period, later supply does not lead to significant effects either on the dry matter production or on alkaloid formation.

The splitting of the complete normal dose into one-third does not bring about such good effects as splitting into one-half and applying these halves, one at the beginning and the other in the middle of the active period of growth. Distribution of nutrients in other ways is not so efficacious.

On alkaloid formation the supply of nitrogen from the beginning is essential to bring about significant effects. The highest amount of alkaloid formation takes place if the plants are supplied with one-half dose of complete fertilizer, one at the beginning of the active growth and the other half in the middle of this period.

Critical period of N is forty days and of P and K seventy days after germination, in the life cycle when their adequate supply is most helpful from growth and alkaloid formation points of view.

REFERENCE

(1) Prasad, S., THIS JOURNAL, **35**, 121(1946).

The Determination of Acetanilid in the Presence of Caffeine*

By ERIC D. ROBINSON and S. C. WERCH

The methods available for the determination of acetanilid in the presence of caffeine are time consuming and complicated. In the proposed method, the sample is first digested with 10 per cent sulfuric acid, forming aniline sulfate, and then the caffeine present is separated by extraction with chloroform. The aniline sulfate is next treated with potassium bromide-bromate solution to form tribromoaniline, and the excess bromide-bromate is titrated with sodium thiosulfate. This procedure gives excellent results with unground as well as ground tablets, is simpler, and permits an average saving in time of about 30 per cent over the other methods with which it has been compared.

THE DETERMINATION of acetanilid in the presence of caffeine is an important procedure in many laboratories. Its most frequent employment, however, probably occurs in the control laboratories of pharmaceutical organizations. Acetanilid and caffeine are often included together in a tablet.

The methods which are employed in the determination of acetanilid in the presence of caffeine are based on the principles of extraction and digestion. They differ from each other only as regards certain possible minor variations in the principles. For example, in the McGaughran method the tablet in question is treated with ether in a Soxhlet extractor, further extracted with chloroform, and then digested with sulfuric acid (1). Or, as suggested in A. O. A. C. (2), one may carry out direct extraction with chloroform and then digest with sulfuric acid. The method described by Wilson and Hilty (3) includes treating the sample first with 2 per cent sulfuric acid, then extracting with chloroform and finally completing the digestion with 1:10 sulfuric acid. All of these methods have been employed successfully in our laboratory. Each of them gives good results. All of them, however, are lengthy.

The method described here grew out of the need to save time. It is less time consuming than any of the methods referred to above, and more accurate in our hands.

METHOD

Place one whole, symmetrical tablet in a small evaporating dish. Add 10 ml. of 10% sulfuric acid and proceed to digest on a water bath for two and one-half hours. From time to time add sufficient water to keep the volume greater than 5 ml. When the odor of acetic acid can no longer be detected the digestion has been completed. Next, wash the material into a long-stemmed Gooch crucible, by means of a fine stream of water. Then filter under reduced pressure. The washing is continued until the liquid comes through water-clean. The filtrate obtained is then poured into a 250-ml. separatory funnel, where the caffeine is extracted with three or four 25-ml. portions of chloroform. The caffeine-chloroform layers, of course, may be saved if desired. The chloroform is evaporated and the caffeine is weighed as anhydrous caffeine. The remaining liquid in the funnel is transferred into a 500-ml. iodine flask. The flask with its contents is gently warmed on the water bath to drive off any chloroform left. To the contents of the flask add exactly 50.00 ml. of standardized 0.1 *N* potassium bromide-potassium bromate solution and 5 ml. of concentrated hydrochloric acid. After allowing the system to stand for five minutes, titrate with standardized 0.1 *N* sodium thiosulfate solution in the presence of potassium iodide and freshly prepared starch indicator.

Although a tablet will serve better as the source of material, a powder mixture can be utilized equally well.

EXPERIMENTAL

The experiments carried out were designed to compare the efficiency of the above method with that of the others previously mentioned. Tablets containing 1.0 gr. of acetanilid and 1.25 gr. of caffeine per tablet, or 20.1% acetanilid by weight, were utilized. Some of the tablets were pulverized, while others were used intact. All experiments were carried out on tablets from the same batch.

The recovery of acetanilid was one criterion for comparison and the time required to carry out the procedure was the other criterion. In a series of ten tests in which method No. 1 was employed, the average recovery was 95.6% and the average time

* Received Dec. 26, 1946 from the Medical Research Division, Plough Inc., Memphis, Tenn.

was six hours and twenty-five minutes. In a series of ten tests in which method No. 2 was employed, the average recovery was 96.1% and the average time seven hours and twenty-five minutes. In a third series of ten tests, where method No. 3 was used, the recovery averaged 98.2% and the time consumed was five hours and five minutes. The proposed method, designated No. 4, gave an average recovery of 99.8% in an equal series of ten tests, while the time consumed was only four hours and fifteen minutes. These averages are enclosed with the accompanying tables, where the values for the tests are presented.

DISCUSSION

In the case where the ground tablets were used, the recoveries in the four methods examined were about the same; 99.4% to 99.6%. However, in the case of unground tablets, a much broader variation in the recoveries was observed. For example, in method No. 1 only 91.8% recovery was obtained. Methods Nos. 2 and 3 also gave lower recoveries for the solid tablet than for the ground one. Method No. 4, however, gave good results both with the ground and unground tablets. Apparently, in the methods where extraction is the first step, lower recoveries are obtained with unground tablets. It is therefore preferable to start with digestion, as carried out in the proposed method.

A perusal of the times required to carry out the different methods points out the greatest advantage embodied in the proposed method. It is readily seen from the tables, that method No. 4 requires practically 35% less time than No. 1, 44% less time than No. 2, and 17% less time than No. 3. The pro-

TABLE I.—METHOD NO. 1 (MCGAUGHRAN)

Part A—Single Ground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.997	99.7	390
2	1.000	0.993	99.3	390
3	1.000	0.990	99.0	390
4	1.000	0.990	99.0	390
5	1.000	0.993	99.3	390
		Av.	99.3	390

Part B—Single Unground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.922	92.2	380
2	1.000	0.900	90.0	380
3	1.000	0.923	92.3	380
4	1.000	0.922	92.2	380
5	1.000	0.922	92.2	380
		Av.	91.8	380

TABLE II.—METHOD NO. 2 (A. O. A. C.)

Part A—Single Ground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.985	98.5	450
2	1.000	0.982	98.2	450
3	1.000	0.982	98.2	450
4	1.000	0.985	98.5	450
5	1.000	0.984	98.4	450
		Av.	98.4	450

Part B—Single Unground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.937	93.7	440
2	1.000	0.935	93.5	440
3	1.000	0.938	93.8	440
4	1.000	0.937	93.7	440
5	1.000	0.937	93.7	440
		Av.	93.7	440

TABLE III.—METHOD NO. 3 (WILSON AND HILTY)

Part A—Single Ground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.985	98.5	310
2	1.000	0.991	99.1	310
3	1.000	0.980	98.0	310
4	1.000	0.992	99.2	310
5	1.000	0.987	98.7	310
		Av.	98.7	310

Part B—Single Unground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.980	98.0	300
2	1.000	0.972	97.2	300
3	1.000	0.975	97.5	300
4	1.000	0.979	97.9	300
5	1.000	0.976	97.6	300
		Av.	97.6	300

TABLE IV.—METHOD NO. 4 (ROBINSON AND WERCH)

Part A—Single Ground Tablets

Sample	Acetanilid Added, Gr.	Acetanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	0.993	99.3	260
2	1.000	0.999	99.9	260
3	1.000	0.990	99.0	260
4	1.000	0.999	99.9	260
5	1.000	0.997	99.7	260
		Av.	99.6	260

Part B—Single Unground Tablets

Sample	Acetanilid Added, Gr.	Actanilid Recovered, Gr.	Recovery, %	Time Consumed, Min.
1	1.000	1.010	101.0	250
2	1.000	1.000	100.0	250
3	1.000	0.998	99.8	250
4	1.000	0.997	99.7	250
5	1.000	0.998	99.8	250
		Av.	100.1	250

posed method is obviously much less time consuming than any of the others.

At this time, the new method has been employed in our laboratory for about six months by different chemists, and found to be best.

CONCLUSIONS

1. A method for the rapid determination of acetanilid has been devised in which excellent recoveries are obtained, both for the ground and unground tablet.

2. The proposed method can be done in much less time than any of the others examined.

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Toxicity of Tyrosine in Normal and Vitamin-Deficient Animals*,†

By GUSTAV J. MARTIN‡

The toxicity of *l*-tyrosine in the rat is greatly increased by riboflavin deficiency but decreased by adding cystine and glycine to the diet.

IN 1941, Martin, *et al.* (1), demonstrated the effect of dopa (dihydroxyphenylalanine) in increasing the blood pressure of dogs rendered hypertensive by the perinephritic technique. This represented the corollary to the treatment of hypertension by tyrosinase introduced by Schroeder (2). Subsequently, Oster and Sorkin (3) reported the effectiveness of dopa in increasing the blood pressure in essential hypertension.

Following this general line of thought, Martin (4) fed diets containing 5 and 10 per cent of *l*-tyrosine. At the 10 per cent tyrosine levels, a syndrome developed originally described by Sullivan, Hess, and Sebrell (5). After approximately two weeks on the diet, there is a blanching of the paws, which appear bloodless, a condition which it is felt corresponds to the "blasser Hochdruck" of Volhard (6). Then the paws become edematous and reddened and the edema extends to the head and shoulders. The animals assume a hunched posture and walk painfully. The eyes become involved; an exudative blepharitis is seen.

Blood pressure determinations were made on these animals at all stages in the syndrome. With few exceptions, it was found that high blood pressure is in evidence at the stage of the blanching of the feet and diminishes thereafter so that determinations on animals showing the marked edema were more frequently within a normal range. During the phase of blanching of feet, the blood pressure averaged 147 mm. for a total of 30 rats, within the range of 135 to 180 mm. The existence of a decarboxylase for tyrosine has been demonstrated (7) and it is suggested that the tyrosine decarboxylase of the kidney forms a concentration of tyramine locally, precipitating a localized ischemia (similar to that produced by constriction of the renal arteries (8)) which accentuated the hypertensive effect. This suggests that where the greatest concentration of the decarboxylase is, there will be the greatest effect of the potentially hyper- or hypotensive agent.

The histopathology of rats fed 10 per cent

* Received Oct. 31, 1946, from The National Drug Company, Philadelphia, Pa.
† Presented in part before the 105th Meeting of the American Chemical Society in Cleveland, April, 1944.
‡ Research Director, The National Drug Company.

tyrosine (9) and the biochemical findings (10) extended the significance of these observations. The rats show degenerative, necrotizing, and fibrosing changes of the pancreas, affecting primarily the exocrinic tissue, but leading ultimately to extensive destruction of the exocrinic and endocrinic tissues. In the kidneys additional degenerative and necrotizing lesions are found, which apparently have their genesis in the excretion of toxic material. The arterioles of the brain, the heart, the lungs, and the kidneys exhibit swelling and hyalinization of the media and occasionally proliferation of the intima, associated with focal hemorrhages, necroses, and glia cell accumulations in the brain. The blood picture (10) showed a hyperglycemia resembling diabetes.

Proceeding with the theory that abnormal amino acid metabolism was involved in the etiology of those cases of essential hypertension characterized by increased blood pressure following the injection of dopa, it became necessary to seek a sensitizing force since the amount of tyrosine in the diet so far exceeded any possible concentration of this amino acid in the diet of the human being. One of the probabilities presenting itself was that of a superimposed vitamin deficiency.

EXPERIMENTAL

The various diets used were modifications of the standard diet prepared by omitting the factor under consideration. The standard diet had the following composition: Vitamin-free casein, 18.0%; sucrose, 67.0%; salts, 4.0%; butter fat, 9.0%; and cod-liver oil, 2.0%. To this were added thiamine hydrochloride, 5.0 mg.; riboflavin, 10.0 mg.; pyridoxine, 5.0 mg.; nicotinic acid, 100 mg.; calcium pantothenate, 100 mg.; choline chloride, 200 mg.; inositol, 200 mg.; and *p*-aminobenzoic acid, 100 mg. Tyrosine was added to this basic composition replacing an equivalent amount by weight of sucrose. Folic acid and biotin do not complicate a diet of this type unless sulfonamides are incorporated.

Rats were placed on diets containing amounts of tyrosine too small to cause any manifestations of disease in animals on vitamin adequate diets. Then, keeping the level of tyrosine constant, various vitamin deficiencies were superimposed. Thiamin, pantothenic acid, folic acid, vitamin A, vitamin D, and pyridoxine deficiencies exerted no detectable effect; however, in the case of the animals kept on riboflavin deficient diets a much greater susceptibility to the effect of tyrosine occurred. Even with very mild deficiencies of riboflavin there was an increase in the toxic effect of the amino acid; the animals were at least four times as sensitive as normally fed rats. 2.5% of tyrosine caused the development of the syndrome where 10% tyrosine was necessary in the rats on vitamin adequate diets. Calder (11) has called attention to a possible relationship of high blood pressure and deficiency of the heat stable B_2 complex. (Table I.)

The basis of the increased sensitivity of the rat to tyrosine in the presence of a riboflavin deficiency is probably the fact that l-amino acid oxidase (12) is a riboflavin containing enzyme. The results here reported are regarded as substantiating this claim. When a deficiency of riboflavin occurs, there is a decreased ability on the part of the body to metabolize tyrosine (customarily by oxidative deamination) and the result is greater concentration of tyrosine exposed to tyrosine decarboxylase, which in turn causes greater localized production of tyramine.

If this hypothesis is correct four further experimental findings should be obtained.

1. *dl*-Tyrosine should not produce effects the equivalent of *l*-tyrosine, because the *d*-tyrosine is more rapidly metabolized. Kögl, *et al.* (13), in

TABLE I.—THE EFFECT OF RIBOFLAVIN DEFICIENCY ON THE TOXICITY OF TYROSINE IN THE RAT

(Value Averages for 20 Rats)

	Months on Diet									
	1		2		3		4		5	
Diet	Av. Weight, Gm.	Deaths, %	Av. Weight, Gm.	Deaths, %	Av. Weight, Gm.	Deaths, %	Av. Weight, Gm.	Deaths, %	Av. Weight, Gm.	Deaths, %
10% Tyrosine	60	70	...	100	...	..	...	..	...	..
10% Tyrosine B_2 Deficient	...	100 in 2 wk.	...	...	...	..	...	..	...	..
5% Tyrosine	84	0	162	0	220	10	220	30	238	50
5% Tyrosine B_2 Deficient	60	70	70	100	...	..	...	..	...	..
2.5% Tyrosine	120	0	182	0	188	0	221	0	250	30
2.5% Tyrosine B_2 Deficient	80	10	86	50	81	70	91	80	100	90
1% Tyrosine	100	0	160	0	181	10	222	10	240	20
1.0% Tyrosine B_2 Deficient	78	0	84	10	88	40	106	70	126	80

studies on the relative ammonia producing effects of the natural and unnatural isomers of amino acids, found that the unnatural isomers produced a somewhat greater increment in urinary ammonia than did the natural isomers. This is in harmony with the fact that most amino acids of the *d*-series are deaminized much more rapidly by tissue slices than are their natural antipodes. *dl*-Tyrosine incorporated into the diet of the rat at 10% did not produce the characteristic syndrome.

tyrosine at 5% by riboflavin deficiency is not duplicated by accentuation of the toxicity of 10% *dl*-tyrosine. Several series were discontinued due to lack of materials.

2. When equivalent amounts by weight of *dl*- and *l*-tyrosine are given to a rat, the blood level of tyrosine following the dosage of *l*-tyrosine should remain higher throughout. Using dosage of 5 mg. per Gm., it was found that after three hours, the blood level of tyrosine in rats given *l*-tyrosine was twice as

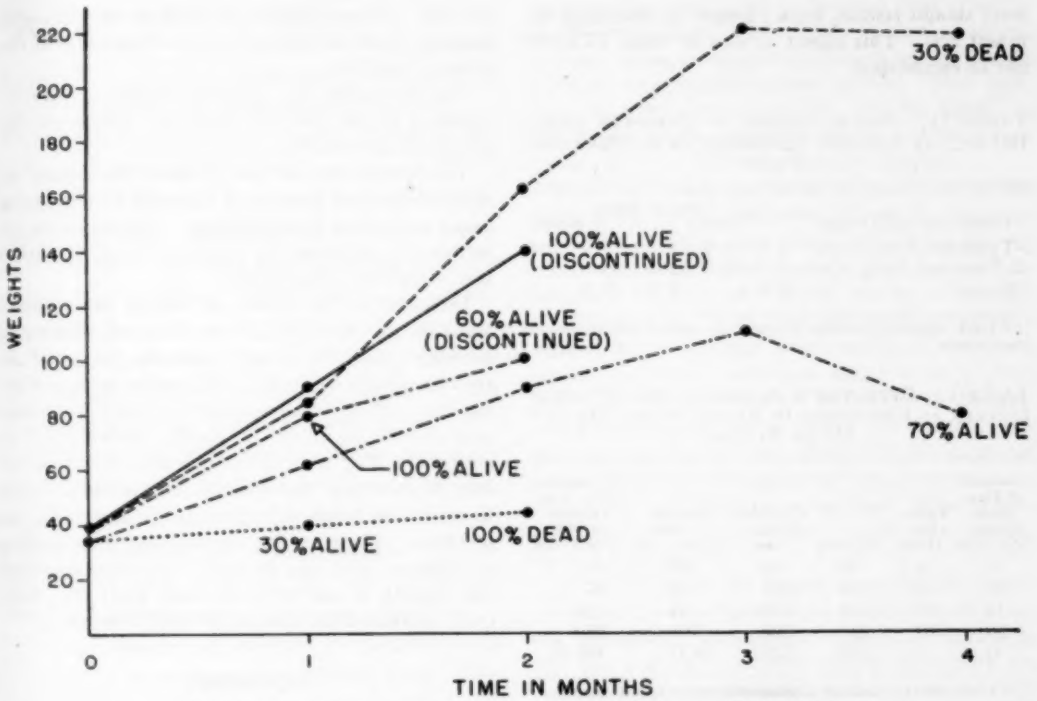

Fig. 1.—Growth curves of rats on various tyrosine containing diets.

Typical sets 10 rats each.
5% *l*-Tyrosine - - - - - - - -
5% *l*-Tyrosine, B_2 Def. . . . . .
10% *dl*-Tyrosine ————————
10% *dl*-Tyrosine, B_2 Def. . . - . - . - . - . - .
B_2 Def. . - . - . - . - .

TABLE II.—BLOOD LEVELS OF TYROSINE (MG./100 ML.) AT VARYING TIME INTERVALS IN NORMAL RATS

Compound	Dose, Mg./Gm.	Time in Hours: 1 Hour				3 Hours				4 Hours				6 Hours				18 Hours			
l-Tyrosine	5.0	4.8	6.7[a]	4.8	6.8[a]	3.9	4.9	4.3	7.3[a]	4.3	7.3[a]	4.9	4.0	..	..	1.8	6.7[a]	1.8	6.6[a]	1.7	1.8[a]	1.6
dl-Tyrosine	5.0	3.0	3.9[a]	3.0	3.9[a]	1.5	2.4	2.8	2.5[a]	2.7	2.5	2.4	1.5	..	..	2.0	2.5[a]	2.0	2.5[a]	2.5	2.0[a]	2.5
Control	..	2.3[b]	1.6[b]	2.2[b]	1.6[b]	1.3	1.5	2.3[b]	1.6[b]	2.2[b]	1.6[b]	1.5	1.3[b]	0.5	0.7	2.3	1.6[b]	2.2[b]	1.6[b]	2.3[b]	1.6[b]	2.2[b]

[a] Given 2 doses at 2-hour intervals. Timed from second dose.
[b] Symbols used to indicate individual experiments. Each value represents result on pooled sample from 5 rats.

Figure 1 shows the accentuation of the syndrome produced by a superimposed riboflavin deficiency. It also shows that the incorporation of 10% *dl*-tyrosine produces an effect during a period of two months exactly paralleling that of 5% *l*-tyrosine. This is theoretically logical as 10% *dl*-tyrosine should contain 5% *l*-tyrosine. In view of this fact, it is strange that the accentuation of the toxicity of *l*-

high as that of rats given *dl*-tyrosine. At eighteen hours, the values are much the same. (Table II.)

3. Urinary excretion of tyrosine should correspond. The findings show that the urinary tyrosine following the administration of *dl*-tyrosine is greater for the earlier periods. The reverse is true of urinary nitrogen. (Table III.)

4. Following large doses of *l*-tyrosine, the blood

TABLE III.—URINARY LEVELS OF NITROGEN AND TYROSINE IN RATS

Compound and Dosage	Determination	Time in Hours			
			4 Hours		16 Hours
l-Tyrosine, 5 mg./Gm.	Urine N, Gm./Kg.	0.59	0.25		1.1
	Mg. tyrosine, 100 cc. Urine		3.9	1.0	
dl-Tyrosine, 5 mg./Gm.	Urine N, Gm./Kg.	0.39	0.16		0.8
	Mg. tyrosine, 100 cc. Urine		9.0	1.9	
Control	Urine N, Gm./Kg.	0.40	0.20		1.2
	Mg. tyrosine, 100 cc. Urine		3.1	0.65	

level should remain higher longer in riboflavin deficient rats. This aspect as seen in Table IV could not be established.

TABLE IV.—BLOOD LEVELS OF TYROSINE (MG./100 CC.) AT VARYING INTERVALS IN B_2 DEFICIENT RATS[a]

Compound and Dosage	Time in Hours				
	3 Hours			4 Hours	
l-Tyrosine, 5 mg./Gm.	4.7	4.7	4.7	1.7	2.0
dl-Tyrosine, 5 mg./Gm.	2.3	3.0	2.3	0.6	1.0
Control	2.0	...	2.0	0.8	1.1

[a] Each value represents average on pooled blood sample from 5 rats.

TABLE V.—EFFECT OF TYROSINE ON THE ACTION OF INSULIN AS REFLECTED IN BLOOD SUGAR (MG./100 ML. OF BLOOD)[a]

Amount of Dextrose Given, Mg./Gm.	Av. Value for #Rats	Control	Tyrosine, 4 Mg./Gm.	Insulin, 0.006 Units/Gm.	Tyrosine, 4 Mg./Gm.; Insulin, 0.006 Units/Gm.
6	8	90	93	56	63
10	10	160	121	73	87
14	10	135	182	103	128
30	10	258	295	340	254
0	6	102	121[b]	69.0	65.8[b]

[a] Time interval insulin administration to blood samples—1 hr.

[b] Rats on 10% tyrosine diet for two months. Large rats—average weight, 250 Gm.

It is suggested that a type of hypertension in man and animal is associated with balance between the intake of certain amino acids, notably tyrosine, and the ability of the organism to dispose of this amino acid. The ability to dispose of the amino acid in the usual manner depends upon the balance between tyrosine decarboxylase, 1-amino acid oxidase and phenol oxidase or tyrosinase. The customary channel of metabolism is oxidative deamination, which involves 1-amino acid oxidase; a riboflavin containing enzyme. Riboflavin deficiency therefore sensitizes to the hypertensive and other toxic effects of tyrosine. It is suggested that the greatest sensitization to tyrosine is not through riboflavin deficiency alone but rather through a multiple deficiency, perhaps the entire B_2 complex. This phase is under study at the present time. Combined pantothenic acid and riboflavin, or pyridoxine and riboflavin deficiencies, had no effect on the toxicity of tyrosine over and above that seen with riboflavin deficiency alone. Further, a consideration of the effect of diet on the concentration and activity of the other enzymes involved will aid in the elucidation of this complex problem.

The possibility that tyrosine interfered with the action of insulin was investigated. The results are presented in Table V.

The interpretations placed upon the results are that interference does occur. Insulin does not bring about as marked hypoglycemia. The series are not sufficiently extensive to warrant a final conclusion but are offered as indicative.

Increases in the levels of certain other amino acids in the diet resulted in lessened toxicity of tyrosine: thus 1% cystine markedly decreased the toxicity of 10% *l*-tyrosine; 5% cystine increased the toxicity; 1% tryptophane and 1% and 5% glutamic acid exerted no effect; N-methyl tyrosine had no action at 0.2%; 5% glycine exerted a significant effect in reducing the toxicity of tyrosine. Riboflavin fed at levels as high as 0.1% and 0.2% had no effect. These results are contrary to the findings of Sullivan, *et al.* (5), in that 5% cystine increased the toxicity of our 10% *l*-tyrosine diet: 1%, however, decreased the toxicity of 10% *l*-tyrosine.

SUMMARY

Riboflavin deficiency markedly increases the toxicity of *l*-tyrosine in the rat. The unnatural form of tyrosine is relatively nontoxic. Variations in blood levels of tyrosine are reported. Cystine and glycine were found to decrease the toxicity of tyrosine.

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Studies on Viburnum. III. The Uterine Sedative Action of Various Fractions*

By I. W. GROTE and MARIBELLE WOODS

Viburnum Prunifolium Fluidextract has been found to exert a uterine sedative action upon the rat uterus *in vitro*. This action appears to reside in amorphous material in the extract instead of in an unidentified crystalline compound isolated by the authors from *Viburnum prunifolium* bark.

WE HAVE MADE extensive use in our laboratories during the past several years of a method of bioassay for uterine sedatives originally suggested by Munch (1). This method consists of a modification of the U. S. P. XII method of bioassay for posterior pituitary. The rat uterus, which gives frequent rhythmic contractions, is used in place of the guinea pig since it is more suitable for measuring sedation. The guinea-pig uterus in contrast gives rather infrequent contractions more suitable for measuring uterine stimulants, such as posterior pituitary. As a standard of sedation, a solution of papaverine hydrochloride is used and the strength of the unknown expressed in terms of gamma of papaverine causing the same sedation as a unit weight of the drug assayed. Multiparous animals selected in the pre-estral stage by vaginal smear are most reliable.

Many assays of various preparations of Viburnum in pure and combined form have been made. Fluidextracts made from authentic root bark of *Viburnum prunifolium* vary in activity from an equivalent of 200 γ of papaverine per cc. up to 1500 γ of papaverine per cc. depending upon the method of preparation as well as the drug used. The sedative activity of alcohol used as a solvent did not interfere with the test results.

EXPERIMENTAL

Using a fluidextract of *Viburnum prunifolium* prepared in the same manner, the work of Evans, Krantz, Harne, and Iwamoto upon fractionation of Viburnum has been repeated (2–4), with the further addition that at each stage of the fractionation procedure, a bioassay by the above method was made of the activity present in the discarded fractions. In general, we find that the steps in the Evans, Iwamoto, Krantz procedure which involve extraction with alcohol show fair recovery of activity, while the discarded residues show but little activity. On the other hand, where water is used as an extraction media, a considerable loss of activity occurs in the gummy, water-insoluble residue. This indicates that the uterine sedative activity resides in the alcohol-soluble gum-like fraction of Viburnum rather than in the water-soluble fraction.

The crystals isolated by us in good yield at the point in the Evans, Iwamoto, Krantz fractionation procedure at which they obtain salicin are not the same compound. Our crystals melt at 186° as compared to a m. p. of 199–201° for the crystals obtained by Evans, *et al.*, and are sweet rather than bitter. Mixed melting points obtained when our crystals are mixed with pure salicin of m. p. 199–201°, as well as when mixed with some of the crystals kindly supplied us by Evans and Krantz, show that our compound is not identical. We have not as yet completed work upon the identification of our compound, but the crystals are inert as to uterine sedative activity when tested in bath concentrations up to 1 part in 2000.

Examination of the charts given in the earlier paper by Evans, Harne, and Krantz, shows that the amorphous, hygroscopic compound melting at 72° has a marked sedative action upon both the human and rat uteri in bath concentrations of 1:6000 and 1:15,000. In their later article, in which they report the isolation of a crystalline compound identified as salicin, their chart shows no sedation on the human uterus *in vitro* when used in concentrations of 1:3000. On the other hand, the aglycone of salicin, salicyl alcohol, commonly called saligenin showed marked sedation when used in a bath concentration of 1:6000. We do not believe these findings would warrant their conclusion that pure salicin alone is the active principle of Viburnum.

We have repeatedly tested the uterine sedative action of salicin *in vitro* in concentrations ranging from 1:100,000 up to 1:1250 without demonstrable activity. On the other hand the aglycone, salicyl alcohol or saligenin, shows marked activity in bath concentrations of 1:5000, while the pink, alcohol-soluble polymerization product of saligenin is markedly active in concentrations of 1:12,500. Furthermore, saligenin and papaverine show quantitative additive uterine sedative effect when added to fluidextracts of Viburnum while salicin does not show this additive effect, since the fluidextract has the same potency as without the added salicin, as shown by Table I.

* Received Sept. 4, 1946, from the Chattanooga Medicine Company, Chattanooga, Tenn.

CONCLUSION

1. Fluidextract of Viburnum prunifolium exerts a definite uterine sedative effect upon the rat uterus *in vitro* and can be assayed in terms of papaverine hydrochloride as a uterine sedative standard.

TABLE I

Preparation	Activity per cc. Expressed as γ Papaverine Hydrochloride
1. Fluidextract Viburnum prunifolium, Sample No. 1	160
2. (1) plus 20 mg. salicin per cc.	170
3. (1) plus 13 mg. salicyl alcohol (saligenin) per cc.	250
4. (1) plus 20 mg. salicyl alcohol (saligenin) per cc.	320
5. (1) plus 200 mg. papaverine HCl per cc.	480
6. Fluidextract Viburnum prunifolium, Sample No. 2	1150
7. (6) plus 20 mg. salicin per cc.	1200
8. (6) plus 20 mg. salicyl alcohol (saligenin) per cc.	1600
9. (6) plus 1 mg. papaverine HCl per cc.	2000

2. The uterine sedative activity of *Viburnum prunifolium* extracts largely resides in the amorphous material present in the extract rather than in the pure crystals that can be obtained from the bark by the method of Evans, *et al.* The crystals obtained by us using this method are not the same as theirs.

3. The aglycone of salicin, namely saligenin, as well as its polymerized, alcohol-soluble forms, possesses definite uterine sedative properties.

4. Recrystallized saligenin, as well as papaverine hydrochloride, shows an additive effect when added to solutions of fluidextract of Viburnum while salicin itself does not do so.

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Book Review

Bacterial Chemistry and Physiology, by JOHN ROGER PORTER. John Wiley and Sons, New York, 1946. x + 1073 pp. 14.5 x 23 cm. Price, $12.

If anyone ever doubted that bacteriology has come of age, an evening spent with *Bacterial Chemistry and Physiology* will dispel any such doubt. Dr. Porter has written a reference book of the highest order showing the more quantitative side of the science of bacteriology.

The first chapter is an excellent review of the physicochemical properties of bacteria and their environment. This chapter of 92 pages discusses succinctly such phenomena as *p*H, colloidal state, surface tension, and adsorption. To many this chapter will be superfluous, to others it will be a welcome review.

Following a chapter on the growth and death of bacteria appear two exceptionally well-written chapters on the effects of physical and chemical agents on bacteria. Many of us pharmacists have been so engrossed in the practical comparison of disinfectants that we may have overlooked the more theoretical dynamics of the disinfection process. Such workers will read with interest the discussion of the kinetics of disinfection and at the same time profit by the summary of the effect of concentration, temperature and surface tension on germicidal action. The activity of the various groups of antiseptics is written in an enlightening fashion.

The chemistry of the principal constituents of bacteria is elucidated in a chapter of 100 pages. In addition to the more common constituents such as proteins, carbohydrates, and fats, the author has given considerable information concerning vitamins, pigments, and pyrogens.

The chapter on bacterial enzymes is recommended to those interested in this phase of the physiology of bacteria. This chapter is the logical precursor to sections on bacterial nutrition and metabolism of carbon and nitrogen compounds. Especially practical are the discussions of the use of bacteria in microbiological assays.

The final chapter is concerned with microbial fermentation. This is made helpful by the generous use of schematic and pictorial flow sheets.

In addition to being well documented, this book appears to be well indexed and includes a microorganism index as well as a general index.

While the greatest use of this compilation will be found at the graduate level, the undergraduate student should refer to it frequently and certainly no teacher who claims to maintain the current viewpoint could be without this book in developing lectures and classroom discussions.—MELVIN W. GREEN.